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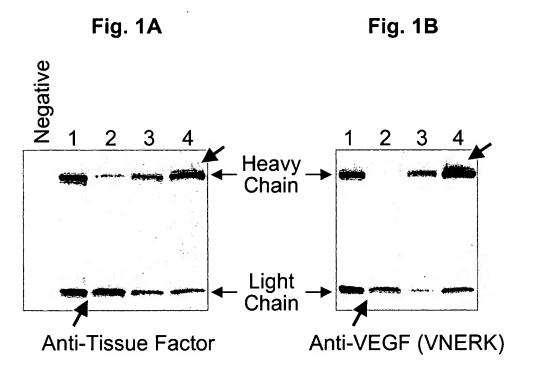
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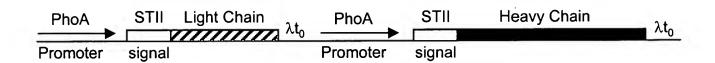
Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK

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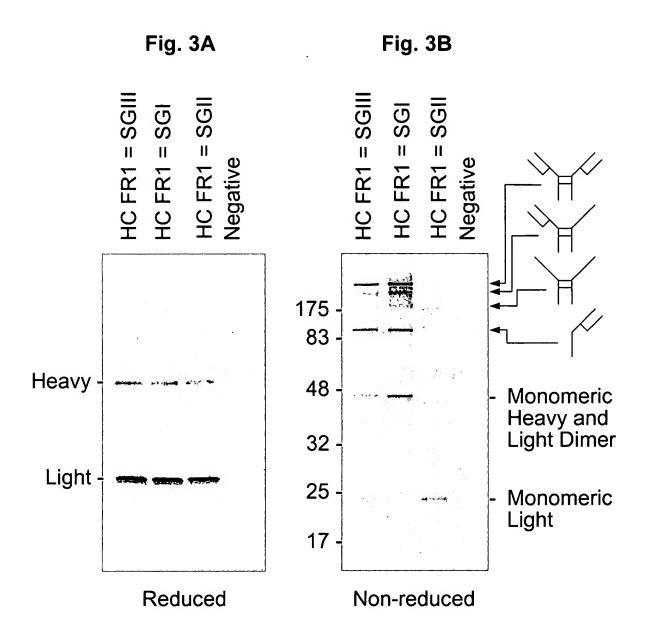
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Fig. 2



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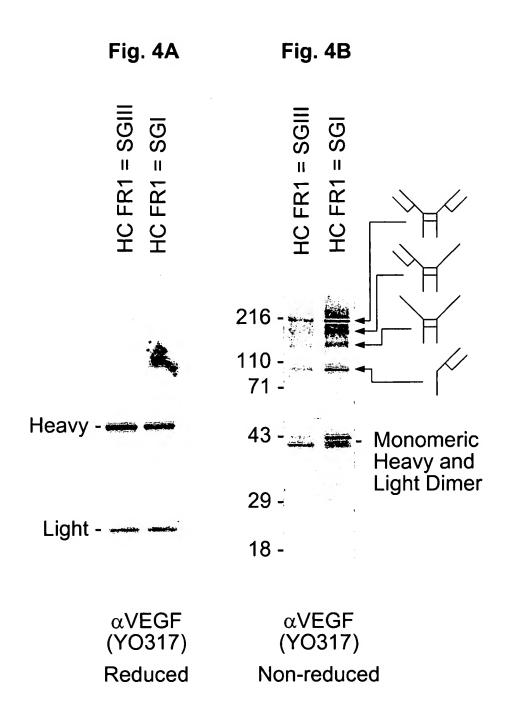




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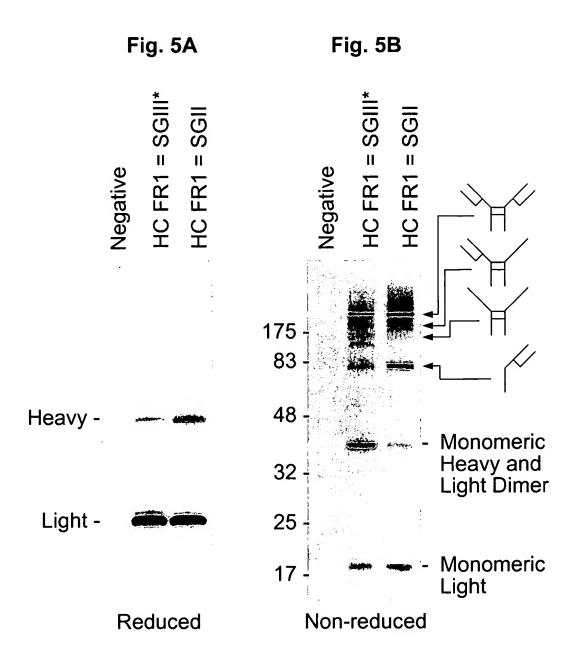
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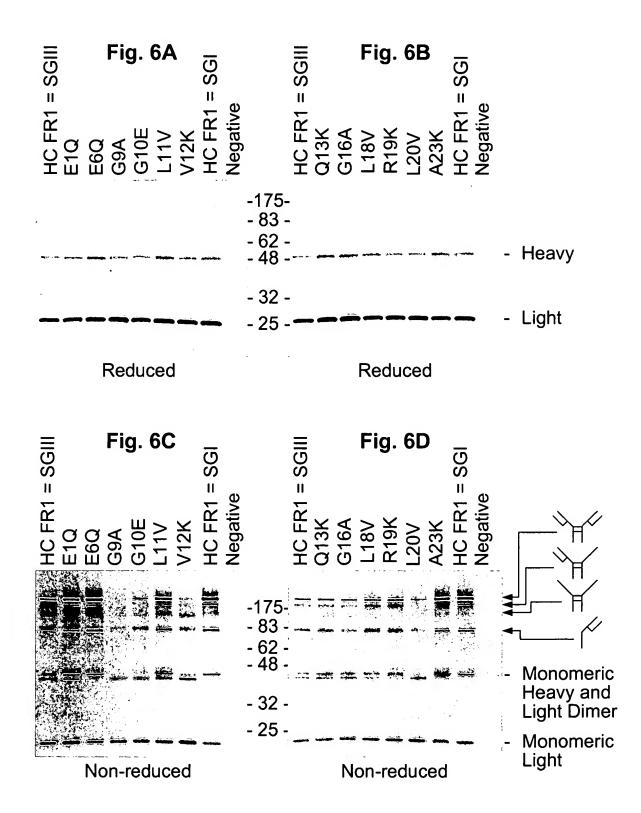
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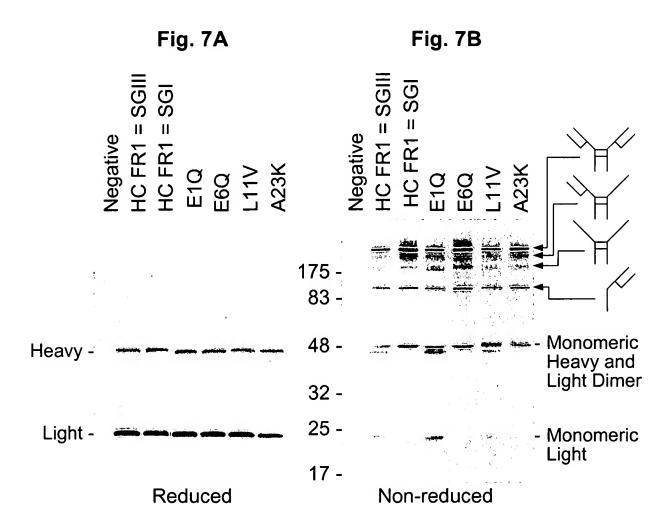
<sup>\*</sup>Includes an A24V change as part of humanization.

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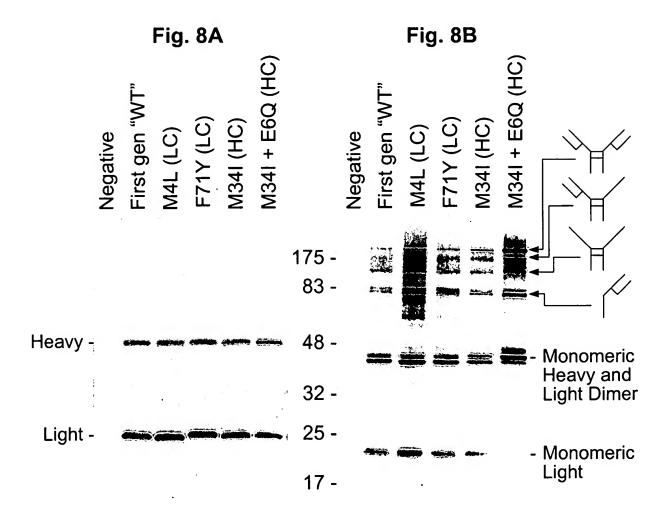


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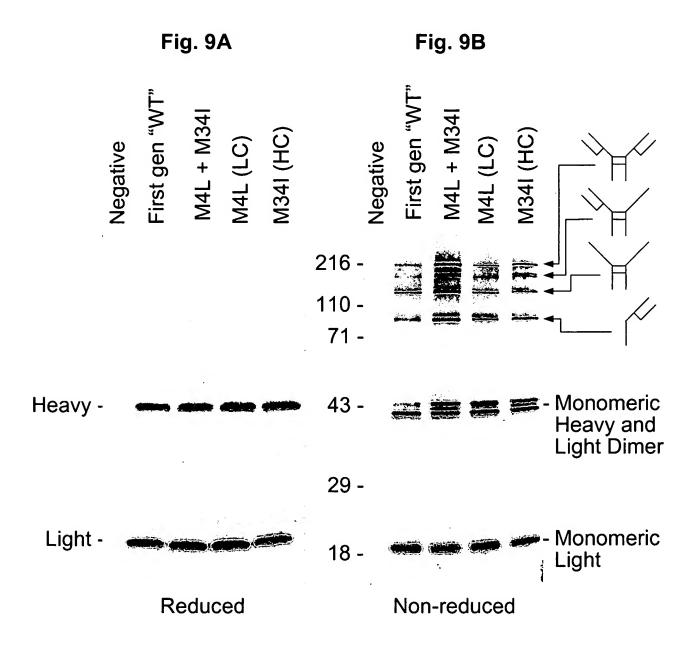
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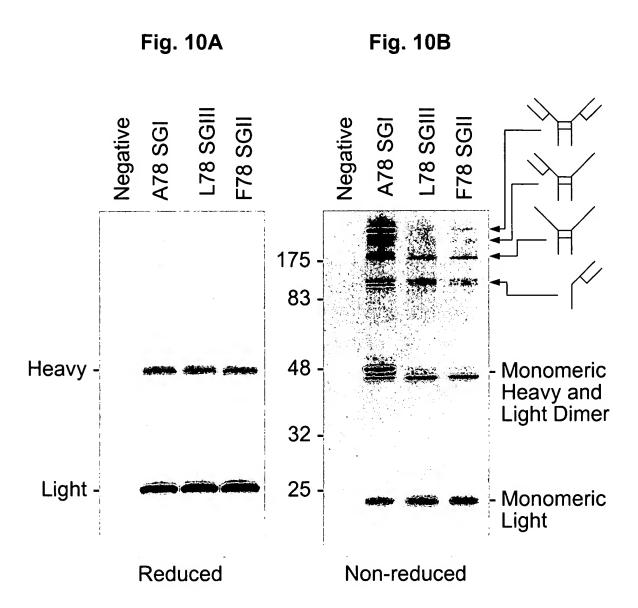


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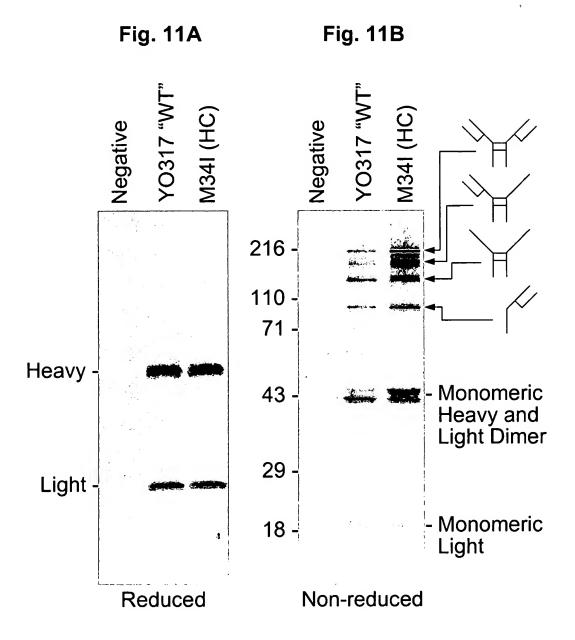
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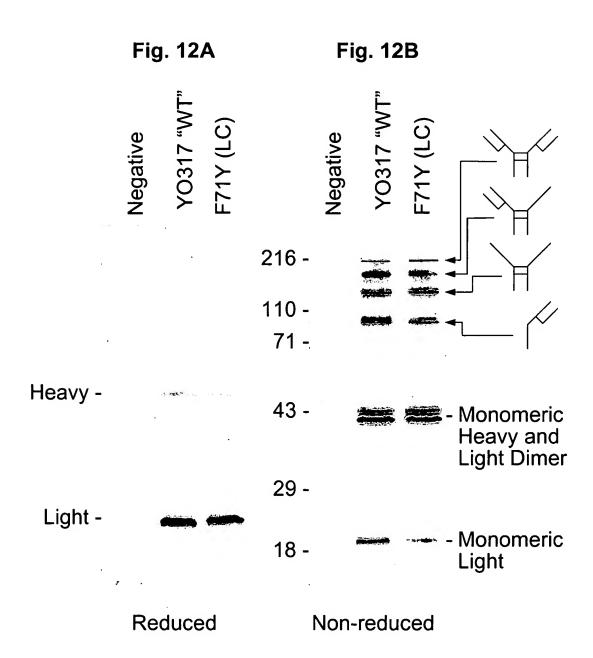
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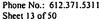


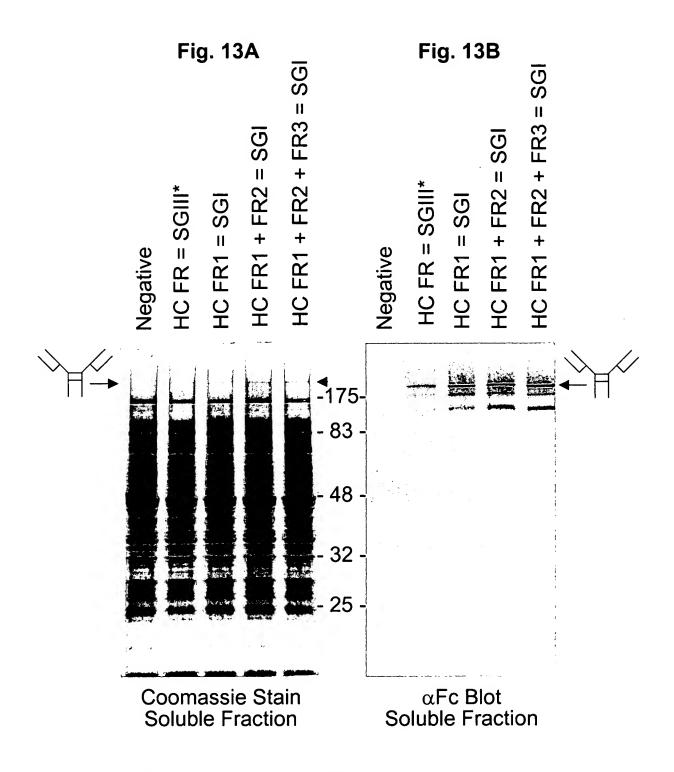
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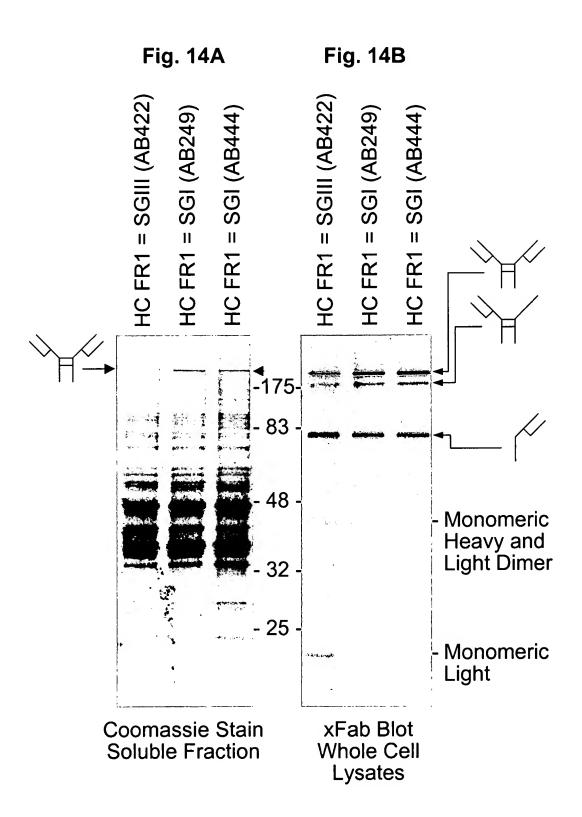
<sup>\*</sup> Except for residues changed during humanization.

Docket No.: 11669.120USUI
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Attorney Name: KATHERINE M. KOWALCHYK

Phone No.: 612.371.5311

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AAAAAGAAGA AGAGTCGAAT CITAAGITGA AGAGGIATGA AACCIATICC ITIAIGICIG IACITITIAG AGIAACGACI CAACAAIAAA ITCGAACGGG ITITICIICI ICTCAGCITA GITGITAIT AAGCITGCCC AAATACAGAC ATGAAAAATC TCATTGCTGA TCTCCATACT TTGGATAAGG GAATTCAACT

gaactgigig cgcaggiaga agcttiggag attaicgica cigcaaigci icgcaataig gcgcaaaaig accaacagcg gtigaitgai caggiagag citgacacac gcgiccaici icgaaaccic iaaiagcagi gacgiiacga agcgiiaiac cgcgiiitac iggiigicgc caaciaacia giccaicicc CIGCAAIGCI ICGCAAIAIG GCGCAAAAIG ACCAACAGCG GAACTGTGTG 101

CCCGCGACAT GCTCCATTIC GGGCTACGGT CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TTICTTCAAT AACTTCGTAG GAGCAGTCAT TTGAAGCATC CTCGTCAGTA GCATTCCTGA CGACGATACG GAGCTGCTGC GCGATTACGT AAAGAAGTTA CCCGATGCCA GGGCGCTGTA CGAGGTAAAG 201

AAAAGTTAAT CITITICAACA GCIGICATAA AGITGICACG GCCGAGACIT ATAGICGCIT IGITITIAIT ITITAAIGIA ITIGTAACTA GTACGCAAGI ITITICAAITIA GAAAAGITIGI CGACAGIAITI ICAACAGIGC CGGCICIGAA IAICAGCGAA ACAAAAATAA AAAAITACAI AAACAITGAI CAIGCGITCA 301

CTTCTTATAG CGTAAAAG AACGTAGATA CAAGCAAAAA AGATAACGAT GTTTGCGCAT GCGACTATAG K N I A F L L A S M F V F S I A T N A Y A D I TCACGTAAAA AGGGTATCTA GAATTATGAA GAAGAATATC GCATTTCTTC TTGCATCTAT GTTCGTTTTT TCTATTGCTA CAAACGCGTA CGCTGATATC anti-VEGF Light Chain AFLL ^STII Signal TIR ~1 AGTGCATTIT ICCCATAGAI CITAATACIT Z X 401

CGGAGACACC CGCTATCCCA GTGGTAGTGG ACGTCGCGTT CAGTCCTATA ATCGTTGATA AATTTGACCA SOI CAGITGACCC AGICCCCGAG CICCCIGICC GCCICIGIGG GCGAIAGGGI CACCAICACC IGCAGCGCAA GICAGGAIAI IAGCAACIAI IIAAACIGGI S ^variable light (VL) cys пΩО CSAS I H œ Ω Q > S GAGGGACAGG Ø ы S GTCAACTGGG TCAGGGGCTC S Д S H H ø

TCTCTCCACT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC AGAGAGGTGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCTG တ S R 단 ß G V P I H S S GCTCCGAAAG TACTGATTTA CTTCACCTCC CGAGGCITTC ATGACTAAAT GAAGTGGAGG ഗ F L I APKV ACCAGGAAAA TGGTCCTTTT s R Д 601 ATCAACAGAA TAGTTGTCTT × o o 9

ITCGCAACIT AITACTGICA ACAGIATAGC ACCGIGCCGI GGACGITIGG ACAGGGIACC TGTCCCATGG უ თ CGGTCTTCTG AAGCGTTGAA TAATGACAGT TGTCATATCG TGGCACGGCA CCTGCAAACC
PEDFATYCOQQXSTVPWTFG ^Variable light cys GCCAGAAGAC GACTGGTAGT CGTCAGACGT GCAGTCTGCA ø H ß CTGACCATCA H GGATTTCACT CCTAAAGTGA H Ŀ Ω 701

)

GGIAGACAGA AGIAGAAGGG CGGIAGACIA CICGICAACI IIAGACCIIG ACGAAGACAA CACACGGACG GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT GTGTGCCTGC V C L A S V E E တ 0 L X S Д CCATCTGTCT TCATCTTCCC н Œ > S Δ, 801 AAGGTGGAGA TCAAACGAAC TGTGGCTGCA Trccaccic Agiliectic Acacceacer K > 2 > × 126

Constant Light cys^

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Phone No.: 612.371.5311

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TCCTGTCGTT GTCACAGAGC GCCTGCGAAG TCACCCATCA CAGTGTCTCG Œ GGTGGATAAC GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GGTCCTCTCA ഗ ы o GCCCATTGAG CAAAGTCTAC S z ტ AAAGCAGACT ACGAGAACA CCACCTATTG CGGGAGGTTA TITCGICIGA IGCICITIGI S o A L z O > TACAGTGGAA GACGCTGAGC ATGTCACCTT \* \* α CTATCCCAGA GAGGCCAAAG CICCGGITIC > GCAGCACCCT E A K TACAGCCTCA GATAGGGTCT Y P R 901 TGAATAACTT ACTIATIGAA GGACAGCACC N N F 160

CATCGTGGCG AGCTCGGTAC CCGGGGATCT AGGCCTAACG ഗ Ö \*Constant Light cys GITICAGAIG CGGACGCTIC AGIGGGIAGI K V Y A C E V T H Q K V Y H H ы × Ω X X CTGCGACTCG S H H CGTCGTGGGA CAACAGGGGA S ATGTCGGAGT CAAAGAGCTT CCTGTCGTGG TCGCCCGTCA S Ω 1101

CAGTGTTAAT TAAATCCTCT ACGCCGGACG CATCGTGGCG AGCTCGGTAC CCGGGGGATCT AGGCCTAACG CTCACAATTA ATTTAGGAGA TGCGGCCTGC GTAGCACCGC TCGAGCCATG GGCCCCTAGA TCCGGATTGC start lambda to terminator^ cys to bind heavy 0 U ω GITICICGAA GITGICCCCT <u>ن</u> د z S × AGCGGGCAGT S 226

CGCCTGCGCG TAGAGCTTAC TTGACACACG CGTCCATCTT CGAAACCTCT AATAGCAGTG ACGTTACGAA TTATCGICAC IGCAAIGCIT GCTTTGGAGA TITIAINGIL GCCGACGCGC AICTCGAAIG AACTGIGIGC GCAGGIAGAA GAGCCAACGG CGGCCCGCAA AAAATAACAA GCCGGGCGTT CICGGITGCC 1201

^end lambda to terminator

CGCAATATGG CGCAAAATGA CCAACAGCGG TTGATTGATC AGGTAGAGGG GGCGCTGTAC GAGGTAAAGC CCGATGCCAG CATTCCTGAC GACGATACGG GCGTTATACC GCGTTTTACT GGTTGTCGCC AACTAACTAG TCCATCTCCC CCGCGACATG CTCCATTTCG GGCTACGGTC GTAAGGACTG CTGCTATGCC CGCAATATGG 1301

TCGACGACGC GCTAATGCAT TICTICAATA ACTICGIAGG AGCAGICAIT TITCAATTAG AAAAGTIGIC GACAGIAITI CAACAGIGCC GGCICTGAAI CGATTACGTA AAGAAGTTAT TGAAGCATCC TCGTCAGTAA AAAGTTAATC TTTTCAACAG CTGTCATAAA GTTGTCACGG AGCTGCTGCG 1401

GITITITATIT ITTAATGTAT TIGIAACTAG TACGCAAGTI CACGTAAAAA GGGTATCTAG AATTATGAAG AAGAATATCG CAITITCTTCT GTAAAGAAGA TTCTTATAGC 'STII Signal TIR-1 ATCAGCGAAA CAAAAATAAA AAATTACATA AACATTGATC ATGCGTTCAA GTGCATTTTT CCCATAGATC TTAATACTTC X 1501 TAGTCGCTTT

GAGTGAGGCA CTCACTCCGT œ ы AAACGCGTAC GCTGAGGTTC AGCTGGTGGA GTCTGGCGGT GGCCTGGTGC AGCCAGGGGG TCGGTCCCCC CCGGACCACG Ø G L anti-VEGF heavy chain (VNERK version) TITIGGGCATG CGACTCCAAG TCGACCACCT CAGACCGCCA S G LVE A E V O × A z TECATCTATE ITCETTITIT CTAITGCTAC A S M F V F S I A T Σ S 4 2

ACCTAATTGT ACCAACTATG GTATAAACTG GGTCCGTCAG GCCCCGGGTA AGGGCCTGGA ATGGGTTGGA TACCCAACCT Ö 3 TCCCGGACCT 闰 ч Ö TGGTTGATAC CATATTTGAC CCAGGCAGTC CGGGGCCCAT AACAGGACAC GTCGAAGACC GATATGGAAG CTATACCTTC Y T F ^Variable Heavy (VH) cys 1701 TTGTCCTGTG CAGCTTCTGG ა დ 43

ACTTGTCGGA TGAACAGCCT z ATGGACGTCT TACCTGCAGA Σ o × TTCGTGTCGT AAGCACAGCA K ۲ S GGATATGGCC ACTTGGCTGG ATACGACGCC TAAAGTTTGC AGCAAAGTGA AAAAGAATC TGTGGAGGTT Y T G E P T Y A A D F K R R F T F S L D T S K ATTICAAACG ICGITICACI TITITCITIAG ACACCICCAA TATGCTGCGG TGAACCGACC CCTATACCGG 1801 77

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CACTGGTATT TCGACGTCTG GGGTCAAGGA CCCAGTTCCT Ö CGCCTTCTCG GTGACCATAA AGCTGCAGAC R K S H W Y F D V W GACACTGCCG TCTATTACTG TGCAAAGTAC CCGCACTATT ATGTGAACGA GCGGAAGAGC GGCGTGATAA TACACTTGCT N > > ь н х CTGTGACGGC AGATAATGAC ACGTTTCATG × C A K \*VH CY8 × K ۲ 1901 GCGCGCTGAG CGCGCGACTC ø œ

GECCTCCACC AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG GCCCTGGGCT CCGGAGGTGG TTCCCGGGTA GCCAGAAGGG GGACCGTGGGG AGGAGGTTCT CGTGGAGACC CCCGTGTCGC CGGGACCCGA Constant Heavy 1 (CH1) cys^ GGCAGAGGAG CCGTCTCCTC S S TGGGACCAGT 2001 ACCCTGGTCA > ы H 143

GGACTACTTC CCCGAACCGG TGACGGTGTC GTGGAACTCA GGCGCCCTGA CCAGGGGGT GCACACCTTC CCGGCTGTCC TACAGTCCTC GGGCTTGGCC ACTGCCACAG CACCTTGAGT CCGCGGGACT GGTCGCCGCA CGTGTGGAAG GGCCGACAGG ATGTCAGGAG s S P A V H × > ც ഗ A L v ഗ z z S > H ک م 田田 CCTGATGAAG [14 × O 2101 GCCTGGTCAA CGGACCAGTT L V K 177

GCGTGGTGAC TGTGCCCTCT AGCAGCTTGG GCACCCAGAC CTACATCTGC AACGTGAATC ACAAGCCCAG CAACACCAAG GTTGTGGTTC H z TCCTGAGATG AGGGAGTCGT CGCACCACTG ACACGGGAGA TCGTCGAACC CGTGGGTCTG GATGTAGACG TTGCACTTAG TGTTCGGGTC G L Y S L S S V V T V P S S L G T O T Y I C N V N H K P S сні сув TCCCTCAGCA 2201 AGGACTCTAC

AAGGAGAAGG TTCCTCTTCC F L CAAATCTTGT GACAAAACTC ACACATGCCC ACGGTGCCCA GCACCTGAAC TCCTGGGGGG ACCGTCAGTC TGGCAGTCAG P S V CACCTGTTCT ITCAACTCGG GTTIAGAACA CTGTTTTGAG TGTGTACGGG TGGCACGGGT CGTGGACTTG AGGACCCCCC

V D K K V E P K S C D K T H T C P P C P A P E L L G G

Cys to bind light chain hinge cys hinge cys GTGGACAAGA AAGTTGAGCC

CAAGGACACC CICAIGAICI CCCGGACCCC IGAGGICACA IGCGIGGIGG IGGACGIGAG CCACGAAGAC CCIGAGGICA AGIICAACIG GAGTACTAGA GGGCCTGGGG ACTCCAGTGT ACGCACCACC ACCTGCACTC GGTGCTTCTG GGACTCCAGT TCAAGTTGAC PEVK Ω H \*Constant Heavy 2 (CH2) cys S A Q E V T Д ۲ 2 ß н E GITCCIGIGG H Ω × 2401 CCCCAAAACC GGGGTTTTTGG × Δ,

GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CGCAGGAGTG GCAGGACGTG > GCACACCAGT R V V S GCCCCCTCC TCGTCATGTT GTCGTGCATG ; ; တ N X O ធ P R E CCGCACCTCC ACGTATTACG GITCTGTITC X H X z z H 团 > 3 2501 GTACGTGGAC CATGCACCTG Ω ×

CCCGTCGGGG GGCAGCCCC ø ტ GGAGTACAAG TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CAAAGCCAAA GTTTCGGTTT K A K TTTGGTAGAG S н H GGAGGGTCGG GGGTAGCTCT CCTCATGITC ACGITCCAGA GGITGITICG E Y K C K V S N K A TGAATGGCAA GICCIGACCG ACTIACCGIT U 2601 CAGGACTGGC 3 Ω 343

GAIGACCAAG AACCAGGICA GCCIGACCIG CCIGGICAAA GGCIICIAIC CCAGCGACAI TTGGTCCAGT CGGACTGGAC GGACCAGTTT CCGAAGATAG GGTCGCTGTA N Q V S L T C L V K G F Y P S D I ^Constant Heavy 3 (CH3) cys S v S CTACTGGTTC M T X GGTGTACACC CTGCCCCCAT CCCGGGAAGA GACGGGGGTA GGGCCCTTCT Œ ы œ S Д I. CCACATGTGG H × > 2701 GAGAACCACA CTCTTGGTGT o Д Œ 377

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Attorney Name: KATHERINE M. KOWALCHYK

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GTCGTTCGAG TCTTCCTCTA CAGCAAGCTC GAGAGGGACA CICICCCIGI LSI × CCICITICITIC AIGITICIDED GCGAGGGCA CGACCIGAGG CIGCCGAGGA AGAAGGAGAT CGTCTTCTCG GCAGAAGAGC ഗ 8 GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC GACGGCTCCT CATGCTCCGT GATGCATGAG GCTCTGCACA ACCACTACAC TGGTGATGTG S × v × CGAGACGTGT LHN ഗ Ω ы æ TTGCAGAAGA GTACGAGGCA CTACGTACTC РР ш H Н CSV H X AACGICITCI z N < z ы ACCCTCTCGT TACCCGTCGG CGTCGTCCCC 2801 CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC GCAGCAGGGG Д o o o Ö o 2901 ACCGTGGACA AGAGCAGGTG TGGCACCTGT TCTCGTCCAC 3 回 ß GCGCCACCTC D X A V E > H

3001 CICCEGETAA AIAAGCAIGE GACGECECTA GAGTECETAA EGETEGETIG EGECEGEGE TITTITATIG ITAACICAIG <u>ITIGACAGE</u>T TAICAIEGIA GAGGECEAII IAITEGIACG EIGECGGGAI ETCAGGGAIT GEGAGECEAE GEGGECEGE AAAAAAIAAE AAITGAGIAE AAACIGIEGA AIAGIAGEIA

<u>ب</u>

Д

477

443

^снз сув

'start of tet resistance promoter ^end lambda t0 terminator 'start lambda to terminator

^-35 of promoter

3101 AAGCTITAAT GCGGTAGTTT ATCACAGTTA AATTGCTAAC GCAGTCAGGC ACCGTGTATG AAATCTAACA ATGCGCTCAT CGTCATCCTC GGCACCGTCA TTCGAAATTA CGCCATCAAA TAGTGTCAAT TTAACGALTG CGTCAGTCCG TGGCACATAC TTTAGATTGT TACGCGAGTA GCAGTAGGAG CCGTGGCAGT 'start of tet resistance translation ^-10 region of tet resistance promoter

CCCTGGATGC TGTAGGCATA GGCTTGGTTA TGCCGGTACT GCCGGGCCTC TTGCGGGATA TCGTCCATTC CGACAGCATC GCCAGTCACT ATGGCGTGCT GGGACCTACG ACATCCGTAT CCGAACCAAT ACGGCCATGA CGGCCCGGAG AACGCCCTAT AGCAGGTAAG GCTGTCGTAG CGGTCAGTGA TACCGCACGA 3201

3301

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK Phone No.: 612.371.5311

GTCACAGAGC AGGACAGCAA CAGTGTCTCG TCCTGTCGTT V T E Q D S K

GCCCTCCAAT CGGGTAACTC CCAGGAGAGT CGGGAGGTTA GCCCATTGAG GGTCCTCTCA

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z

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GAGGCCAAAG TACAGTGGAA GGTGGATAAC CTCCGGTTTC ATGTCACCTT CCACCTATTG E A K V Q W K V D N

CTATCCCAGA

901 TGAATAACTT z

ACTIVATIGAA GATAGGGICT

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TCGAA'	TAGAG	TCAGT	GCAAG	GATATY CTATA D I	ACTGG TGACC	TGGGA ACCCT G T	GGTAC CCATG G T	GCCTG
AGAG	CAGG	CTCG	GTAC	CGCT GCGA A 0317)	TTAA AATT L	GTTC CAAG S	ACAG TGTC Q	GTGT
GAAGA	TTGAT	GCATC	AACTA TTGAT	GCGTA CGCAT A Y ion Y	ACTAT TGATA	ATCCG TAGGC S G	TTTGG PAACC F G	CTGTT
AAAAA	GTTGA	TTGAA	TTTGT	CAAACGCGTA CGCTGGGTTGGCGAT GCGACN N A Y A D (version Y0317)	TAGCA ATCGT S N	TCTGG AGACC S G	GGACG CCTGC T	TGCTT
TGCCC ACGGG	CAGCG	AGTTA	ATGTA	rGCTA ACGAT A T Shain	SATAT CTATA D I	SCTTC CGAAG F	SCCGT CGGCA P W	GGAAC
AAGCT	ACCAA( TGGTT	AAAGAJ TTTCT:	TTTTA	PAGCAAAA AGATAACGAT F V F S I A T Anti-VEGF Light chain	GTCAG CAGTC	TTCTC( AAGAG( S R	ACCGTV TGGCA( T V	AATCT TTAGA
TAAA	AATG	ACGT	TATT	TTTT AAAA F EGF L	GCAA CGTT A S	TCCC PAGGG	TAGC PATCG S	TTGA
GAATICAACT TCTCCATACT TIGGATAAGG AAATACAGAC ATGAAAAATC TCATIGCTGA GTIGTTATTT AAGCTIGCCC AAAAAGAAA AGAGTCGAAT CTIAAGIIGA AGAGGIAIGA AACCIAITCC TITAIGICTG TACTITITAG AGIAACGACT CAACAATAAA TICGAACGGG TITITCTICT ICTCAGCTTA	AGCTTTGGAG ATTATCGTCA CTGCAATGCT TCGCAATATG GCGCAAAATG ACCAACAGGG GTTGATTGAT CAGGTAGAGG TCGAAACCTC TAATAGCAGT GACGTTACGA AGCGTTATAC CGCGTTTTAC TGGTTGTCGC CAACTAACTA GTCCATCTCC	CCCGATGCCA GCATTCCTGA CGACGATACG GAGCTGCTGC GCGATTACGT AAAGAAGTTA TTGAAGCATC CTCGTCAGTA GGGCTACGGT CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TTTCTTCAAT AACTTCGTAG GAGCAGTCAT	GCTGTCATAA AGTTGTCACG GCCGAGACTT ATAGTCGCTT TGTTTTTATT TTTTAATGTA TTTGTAACTA GTACGCAAGT CGACAGTATT TCAACAGTGC CGGCTCTGAA TATCAGCGAA ACAAAATAA AAAATTACAT AAACATTGAT CATGCGTTCA	GAATTATGAA GAAGAATATC GCATTTCTTC TTGCATCTAT GTTCGTTTTT TCTATTGCTA CAAACGCGTA CGCTGATATAC CTTAATACTT CTTCTTATAG CGTAAAGAAG AACGTAGATA CAAGCAAAAA AGATAACGAT GTTTGCGCAT GCGACTATAG M K K N I A F L L A S M F V F S I A T N A Y A D I ^STII Signal TIR ~1	CTCCCTGTCC GCCTCTGTGG GCGATAGGGT CACCATCACC TGCAGGGCAA GTCAGGATAT TAGCAACTAT TTAAACTGGT GAGGGACAGG CGGAGACACC GGCTATCCCA GTGGTAGTGG ACGTCGCGTT CAGTCCTATA ATCGTTGATA AATTTGACCA S L S A S V G D R V T I T C S A S Q D I S N Y L N W Y	ATCAACAGAA ACCAGGAAAA GCTCCGAAAG TACTGATTTA CTTCACCTCC TCTCTCCACT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC TAGTTGTCTT TGGTCCTTTT CGAGGCTTTC ATGACTAAAT GAAGTGGAGG AGAGAGGTGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCTG Q Q K P G K A P K V L I Y F T S S L H S G V P S R F S G S G S G T	701 GGATTTCACT CTGACCATCA GCAGGAAGAC TTCGCAAACTT ATTACTGTCA ACAGTATAGC ACCGTGCCGT GGACGTTTGG ACAGGGTACC CCTAAAGTGA GACTGGTAGT CGTCAGACGT CGGTCTTCTG AAGCGTTGAA TAATGACAGT TGTCATATCG TGGCACGGCA CCTGCAAACC TGTCCATGG 93 D F T L T I S S L Q P E D F A T Y Y C Q Q Y S T V P W T F G Q G T	TGTGGCTGCA CCATCTGTCT TCATCTTCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT GTGTGCCTGC ACACCGACGT GGTAGACAGA AGTAGAAGGG CGGTAGACTA CTCGTCAACT TTAGACCTTG ACGAAGACAA CACACGGACG V A A C V V C I I.
CTGA (	TATG (	CTGC (	GCTT C	CTAT (GATA (M)	CACC COTGG I	CACT (GTGA )	GTCA CAGT	TGAT
CATTG	CGCAA	RECTG	TAGTC PATCAG	TTGCATC	SACCAT STGGTA T I	CTCTC AGAGAG S L	ATTACT FAATGA Y C	SCCATC SGGTAG
AATC 1	ACGA A	TACG CATGC C	ACTT A	CTTC 1 GAAG 7 L L	GGGT C	CTCC 1 GAGG 1	ACTT / TGAA 1 T Y	TCCC C
TGAAA	TGCAA	GACGA	CCGAG	GAAGAATATC GCATTTCTTC CTTCTTATAG CGTAAAGAAG K N I A F L L Signal TIR ~1	CGATA GCTAT	TTCAC AAGTG	TCGCA AGCGT	CATCT
GAC A	TCA C	TGA C	ACG G	ATC GATAGC I A TIR ~	rrgg g	TTA C	GAC T	STCT T
ATACA FTATGI	FTATCG	CATTCC	STIGIC	AAGAAT FTCTTA K N Ignal	SCTCTG SGAGAC S V	ACTGAT FGACTP L I	CCAGAA 3GTCTT P E	CATCTC
AGG AV	SAG AT	SCA GO	FAA AC	TATGAA GAATACTT CTAR N K 1	TCC GC AGG CC	AAG TI TTC AI	SCA GO CGT CO	GCA CC
GGATA	CTTTG	CGATG	TGTCA	ATTATGA TAATACT: M K ^STI	CCCTG GGGAC	TCCGA AGGCT	AGTCTY TCAGA( S L	TGGCTA
T TT	A AG			ra ga		9 F.	8 F. 8	36 AC 76
CATAC	GGTAG	GTAAA	TCAAC	татст	CCCGA GGGCT P S	GGAAA CCTTT G K	CCATC GGTAG	ACGAA
TCTC	CGCA	CGAG	CTTT	AGGG	AGTC( TCAG( S	ACCA TGGI P	CTGA GACI L I	TCAA AGTT
CAACT	SAACTGTGTG CGCAGGTAGA CTTGACACAC GCGTCCATCT	GGCGCTGTA CGAGGTAAA CCCGCGACAT GCTCCATT	aaaagitaat cititicaaca Iititcaaita gaaaagitgi	taaaa Attet	SACCC CTGGG T Q	CAGAA GTCTT Q K	TCACT AGTGA T	GGAGA
1 GAATTCAACT TCTCCATACT CTTAAGTTGA AGAGGTATGA	101 GAACTGTGTG CGCAGGTAGA CTTGACACAC GCGTCCATCT	201 GGGCGCTGTA CGAGGTAAAG CCCGCGACAT GCTCCATTTC	301 AAAAGTTAAT CTTTTCAACA TTTTCAATTA GAAAAGTTGT	401 TCACGTAAAA AGGGTATCTA AGTGCATTTT TCCCATAGAT 1	501 CAGTIGACCC AGTCCCCGAG GTCAACTGGG TCAGGGGCTC 26 Q L T Q S P S	601 ATCAACAGAA ACCAGGAAAA TAGTTGTCTT TGGTCCTTTT 60 Q Q K P G K	GGATT CCTAA D F	801 AAGGTGGAGA TCAAACGAAC TTCCACCTCT AGTTTGCTTG
н	101	201	301	101	501	601 7	701	801

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK Phone No.: 612.371.5311

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CCCGGACTCG TCCGGATTGC GGCCTGAGC Ы CAAAGICIAC GCCIGCGAAG ICACCCAICA TITCGICIGA IGCICITIGI GITICAGAIG CGGACGCIIC AGIGGGIAGI GCCCCTAGA CCGGGGGATCT > AGCTCGGTAC TCGAGCCATG ш ບ CATCGTGGCG GTAGCACCGC K V Y AAAGCAGACT ACGAGAAACA ACGCCGGACG TGCGGCCTGC × ы TAAATCCTCT ATTTAGGAGA K A CTCACAATTA CTGCGACTCG GAGTGTTAAT GACGCTGAGC ഗ Ы E C CGTCGTGGGA GCAGCACCCT CAACAGGGGA GTIGICCCCT Ö z z S GGACAGCACC TACAGCCTCA ccrercered Argredgagr CAAAGAGCTT GTTTCTCGAA S Н တ × TCGCCCGTCA AGCGGGCAGT SPV ഗ 1101 226 1001

FIG. 16B

TGCAATGCTT GAGCCAACGG CGGCCCGCAA AAAATAACAA CGGCTGCGGG TAGAGCTTAC TTGACACACG CGTCCATCTT CGAAACCTCT AATAGCAGTG ACGTTACGAA TTATCGTCAC GCTTTGGAGA GCAGGTAGAA AACTGTGTGC GCCGACGCGC ATCTCGAATG TTTTATTGTT GCCGGGCGTT CTCGGTTGCC

GACGATACGG CTGCTATGCC CGCAATATGG CGCAAAATGA CCAACAGGGG TTGATTGATC AGGTAGAGGG GGCGCTGTAC GAGGTAAAGC CCGATGCCAG CATTCCTGAC GCGTTATACC GCGTTTTACT GGTTGTCGCC AACTAACTAG TCCATCTCCC CCGCGACATG CTCCATTTCG GGCTACGGTC GTAAGGACTG CGCAATATGG 1301

CCGAGACTTA AAGAAGTTAT TGAAGCATCC TCGTCAGTAA AAAGTTAATC TTTTCAACAG CTGTCATAAA GTTGTCACGG TTCTTCAATA ACTTCGTAGG AGCAGTCATT TTTCAATTAG AAAAGTTGTC GACAGTATTT CAACAGTGCC CGATTACGTA GCTAATGCAT AGCTGCTGCG rcgacgacgc 1401

TITAAIGIAI TIGIAACTAG TACGCAAGII CACGIAAAAA GGGIAICTAG AATTAIGAAG AAGAATAICG CAITICITCI GTAAAGAAGA ATCAGCGAAA CAAAAATAAA AAATTACATA AACATTGATC ATGCGTTCAA GTGCATTTTT CCCATAGATC TTAATACTTC TTCTTATAGC KNIA M GTTTTTATTT TAGTCGCTTT 1501

STII Signal TIR-1

CTCACTCCGT GAGTGAGGCA ч S TECATCTATE TTCGTTTTTT CTATTGCTAC AAACGCGTAC GCTGAGGTTC AGCTGGTGA GTCTGGCGGT GGCCTGGTGC AGCCAGGGGG TCGGTCCCCC Ö Д TITICGCCATG CGACTCCAAG TCGACCACCT CAGACCGCCA CCGGACCACG o G L V Anti-VEGF Heavy Chain (version Y0317) <u>ი</u> S L V E E V Ø A K z GATAACGATG Η ø AAGCAAAAAA S Œ > Œ ACGTAGATAC Σ ഗ 4 1601

TGGATTAACA ACCTAATTGT CTACGACTIC ACGCACTACG GTATGAACTG GGTCCGTCAG GCCCCGGGTA AGGCCCTGGA ATGGGTTGGA TACCCAACCT × TCCCGGACCT Œ Ö × CGGGGCCCAT ტ Д Ø CATACTTGAC CCAGGCAGTC o a > NN TGCGTGATGC Ö T H Y GATGCTGAAG [2, Ω × CAGCTTCTGG GTCGAAGACC G ຜ TIGICCIGIG **AACAGGACAC** 1701

TGAACAGCCT ACTIGICGGA ы z ATGGACGTCT TACCTGCAGA T X AAGCACAGCA TTCGTGTCGT 4 H S TCGTTTCACT TTTTCTTTAG ACACCTCCAA TGTGGAGGTT × ß H AAAAGAAATC SLD AGCAAAGTGA FI T ద TATGCTGCGG ATTTCAAACG ATACGACGCC TAAAGTTTGC ద × Ŀ Ω ø Y A TGAACCGACC ACTTGGCTGG Δ, ы GGATATGGCC CCTATACCGG Ö × 1801 77 AACCCTGGTC TTGGGACCAG ы GGGGTCAAGG CCCCAGTTCC o ဗ F D V W TTCGACGTCT CCACTGGTAT GGTGACCATA н м CCGTACTATT ATGGGACGAG TACCCTGCTC T S ტ GGCATGATAA 1 P Y Y Y TCTATTACTG TGCAAAGTAC AGATAATGAC ACGTTTCATG K CTGTGACGGC A V GACACTGCCG H GCGCGCTGAG CGCGCGACTC Ø œ 1901

ACGGACCAGT GGCCCTGGGC CCGGGACCCG Ö ы ø GGGCCACAGC CCCCGTGTCG Þ H Ö TCGTGGAGAC Ö AGCACCTCTG ഗ H S CTCCTCCAAG GAGGAGGTTC × တ တ GGGACCGTGG CCCTGGCACC A ч AGCCAGAAGG CAAGGCCCA TCGGTCTTCC Д [± > ഗ GTTCCCGGGT Д Ö × CGGCCTCCAC GCCGGAGGTG H ß Þ TGGCAGAGGA ACCGTCTCCT ß S > Н 2001 143

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
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2101	AGGACTACTT	AGGACTACTT CCCCGAACCG	GTGACGGTGT	CGTGGAACTC	AGGCGCCCTG	2101 AGGACTACTT CCCCGAACCG GTGACGGTGT CGTGGAACTC AGGCGCCCTG ACCAGCGGCG TGCACACTT CCCGGCTGTC CTACAGTCCT CAGGACTCTA TCCTGATGAA GAGGCTTTGCC CACTGCCACA GCACCTTGAG TCGGCGGAC TGGTCGCGC ACGTGTGAAA GAGGCGAAG GATGTCAAGA GTCCTGAGAT	TGCACACCTT	CCCGGCTGTC	CTACAGTCCT	CAGGACTCTA
177		<u>а</u>	V T V	S N	G A L	T S G V	H T F	P A V	r o s s	G L Y
2201	CTCCCTCAGC	AGCGTGGTGA	CTGTGCCCTC	TAGCAGCTTG	GGCACCCAGA	2201 CTCCCTCAGC AGGRIGGIGA CTGTGCCCTC TAGCAGCTTG GGCACCCAGA CCTACATCTG CAACGGAAT CACAAGCCCA GCAACAAA GAGGAAGTG TCGCACCACT GACACGAGA ATCGTCGAAC CCGTGGGTCT GGATGTAGAC GTTGCACTTA GTGTTCGGGT CGTTGTGTGT	CAACGTGAAT	CACAAGCCCA		GGTGGACAAG CCACCTGTTC
210	S T S	S v T	V P S	S S	G T Q T	Y I C	N N	н к Р S		V D K
2301	AAAGTTGAGC	CCAAATCTTG	TGACAAAACT	CACACATGCC	CACCGTGCCC	2301 AAAGTIGAGC CCAAATCIIG IGACAAAACT CACACAIGCC CACCGIGCCC AGCACCIGAA CICCIGGGGG GACCGICAGT CITCCICITC CCCCCAAAAC ITTCAACICG GGITIAAAAC ACIGITITGA GICIGTACGG GIGGCACGG ICGIGGACTI GAGGACCCC CIGGCAGTCA GAAGGACAAG GGGGGITIIG	CTCCTGGGGG	GACCGTCAGT	CITCCTCTTC	CCCCCAAAAC
243		S S	D K T	нтср	ъсъ	A P E	LLGG	P S V	7 7	РРКР
2401	2401 CCAAGGACAC	CCTCATGATC	TCCCGGACCC	CTGAGGTCAC	ATGCGTGGTG	CCAAGGACAC CCTCATGATC TCCCGGACCC CTGAGGTCAC ATGCGTGGTG GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT	GCCACGAAGA	CCCTGAGGTC	CCCTGAGGTC AAGTTCAACT	GGTACGTGGA
277	_	L M I	S R T P	E V T	C V V	V D V S	H E D	P E V		Y V D
2501	CGCCGTGGAG	GTGCATAATG	CCAAGACAAA	GCCGCGGGAG	GAGCAGTACA	2501 GGGGTGGAG GTGCATAATG CCAAGACAAA GCGGGGAG GAGCAGTACA ACAGCAGTA CGGTGTGGTC AGGGTCCTCA CGGTCCTGCA GCGTCCTTCA CGGAGGAGT GCGAGGAGT GCGAGAGGAGT GCGAGGAGT GCGAGGAGT GCGAGGAGT GCGAGAGGAGT GCGAGGAGT GCGAGAGGAGT GCGAGAGGAGT GCGAGGAGT GCGAGGAGT GCGAGGAGT GCGAGGAGT GCGAGAGAGT GCGAGAGAGAGT GCGAGAGAGT GCAGAGAGT GCAGAGAGAGT GCAGAGAGAGAGT GCGAGAGAGAGT GCGAGAGAGAGT GCGAGAGAGT GCGAGAGAGAGT GCGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAG	CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA	AGCGTCCTCA		CCAGGACTGG
310	ы >	V H N A	K T K	ਰ ਲ ਜ਼	E O Y	STY	R V V	SVLT		Q D W
2601	2601 CTGAATGGCA AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC GACTTACCGT TCCTCATGTT CACGTTCCAG AGGTTGTTTC GGAAGGGTCG	CTGAATGGCA AGGAGTACAA GACTTACCGT TCCTCATGTT	GTGCAAGGTC	TCCAACAAAG	CCCTCCCAGC	CCCCATCGAG	AAAACCATCT CCAAAGCCAA AGGGCAGCCC TTTTTGGTAGA GGTTTCGGTT TCCCGTCGGG	CCAAAGCCAA		CGAGAACCAC
343		E Y K		S N K	L P A		KTIS	K A K		R E P O
2701	2701 AGGIGTACAC CCTGCCCCCA TCCCGGGAAG AGATGACCAA TCCACATGTG GOALCGGGGT AGGCCCTTTC TCTALTGGTT	CCTGCCCCCA	TCCCGGGAAG	AGGIGIACAC CCTGCCCCA TCCCGGGAAG AGATGACCAA	GAACCAGGTC	GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGGGACA TCGCCGTGGA	GCCTGGTCAA	AGGCTTCTAT	CCCAGCGACA	TCGCCGTGGA
377		L P P	SREE	MTK		S L T C	L V K	G F Y		A V E
2801	GTGGGAGAGC	AATGGGCAGC	AATGGGCAGC CGGAGAACAA TTACCCGTCG GCCTCTTGTT	AATGGGCAGC CGGAGAACAA CTACAAGACC TTACCCGTCG GCCTCTTGTT GATGTTCTGG	ACGCCTCCCG TGCGGAGGGC	CTACAAGACC ACGCCTCCCG TGCTGGACTC GATGTTCTGG TGCGGAGGGC ACGACCTGAG	CGACGGCTCC	TTCTTCCTCT ACAGCAAGCT AAGAAGGAGA TGTCGTTCGA		CACCGTGGAC
410	32 E3	Z C D	E N	¥ K	т р р v	L D S	S D C	F F L Y		T V D
2901	AAGAGCAGGT	GGCAGCAGGG	GAACGICITC	TCATGCTCCG	TGATGCATGA	2901 AAGAGCAGGT GGCAGGAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA TTCTCGTCCA CCTCGTCC CTTGCAGAAG AGTACGAGGC ACTACTACT CCGACACGTG TTGGTGATGT GCGTCTTCTC GGAGAGGAA AGAGGCCAT	AACCACTACA	CCCAGAAGAG	CCTCTCCCTG	TCTCCGGGTA
443		5 0 0	N V	SCSV	MHE	A L H	NHYT	O K S	LSL	S P G K
3001	AATAAGCATG	CGACGGCCCT	AGAGTCCCTA	AATAAGCATG CGACGGCCCT AGAGTCCCTA AGGCTGGGTT GCCGCCGGGC TTAATACGTAA GCTGCCGGG TTAATACGTAA CGCCCGGA CGCCCCCGA	255522525	3001 AATAAGCATG CGACGGCCCT AGAGTCCCTA AGGCTCGGTT GCCGCCGGGC GTTTTTTATT GTTAACTCAT GTTTGACAGC TTATCATCGA TAAGCTTTAA TTATTCTBAC GTTGCCGGG TTTCAGGGAT TGCGAGCCAA CGCCGCGC CAAAAAAAAA CAAAAAAAAA	GTTAACTCAT	GTTTGACAGC	TTATCATCGA	TAAGCTTTAA
477										
3101	TGCGGTAGTT ACGCCATCAA	TATCACAGTT ATAGTGTCAA	AAATTGCTAA TTTAACGATT	CGCAGTCAGG GCGTCAGTCC	CACCGTGTAT GTGGCACATA	3101 TGGGGTAGIT TATCACAGIT AAATTGCTAA GGCAGTCAGG CACCGTGTAT GAAATCTAAC AATGGGGTCA TGGTCATCCT CGGCACCGTC ACCCTGGATG AGGCCATCAA ATAGTGTCAA TITAACGATT GCGTCAGTCC GTGGCACATA CTTTAGATTG TTACGGGAGT AGCAGTAGGA GCCGTGGCAG TGGGACCTAC ^Start Tet Resistance Coding Sequence	AATGCGCTCA TTACGCGAGT rt Tet Resi	TCGTCATCCT AGCAGTAGGA stance Codi	AC AATGCGCTCA TCGTCATCCT CGGCACCGTC TG TTACGCGAGT AGCAGTAGGA GCCGTGGCAG Start Tet Resistance Coding Sequence	ACCCTGGATG TGGGACCTAC

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
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Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
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3201 CTGTAGGCAT AGGCTTGGTT ATGCCGGTAC TGCCGGGCCT CTTGCGGGAT ATCGTCCATT CCGACAGCAT CGCCAGTCAC TATGGCGTGC TGCTAGCGCT GACATCGCTA GCGTCGCTA GCGGTCAGTA ATACCGCACG ACGATCGCGA

3301

# K

AGAGTCGAAT CTTAAGTIGA AGAGGIAIGA AACCTATICC ITTAIGICIG IACTITITAG AGIAACGACI CAACAAIAAA ITCGAACGGG ITTITCTICI ICTCAGCITA AAAAAGAAGA AAGCTTGCCC GTTGTTATTT TCATTGCTGA AAATACAGAC ATGAAAAATC TTGGATAAGG TCTCCATACT GAATTCAACT

GAACTGTGTG CGCAGGTAGA AGCTTTGGAG ATTATCGTCA CTGCAATGCT TCGCAATATG GCGCAAAATG ACCAACAGGG GTTGATTGAT CAGGTAGAGG CTTGACACAC GCGTCCATCT TCGAAACCTC TAATAGCAGT GACGTTACGA AGCGTTATAC CGCGTTTTAC TGGTTGTCGC CAACTAACTA GTCCATCTCC 101

Inventor: SIMMONS Docket No.: 11669.120USU1

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Attorney Name: KATHERINE M. KOWALCHYK

GGGCGCTGTA CGAGGTAAAG CCCGATGCCA GCATTCCTGA CGACGATACG GAGCTGCTGC GCGATTACGT AAAGAAGTTA TTGAAGCATC CTCGTCAGTA CCCGCGACAT GCTCCATTTC GGGCTACGGT CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TTTCTTCAAT AACTTCGTAG GAGCAGTCAT GGCGCTGTA 201

AAAAGITAAI CITITCAACA GCIGICAIAA AGITGICACG GCCGAGACII AIAGICGCII IGIITITIAII ITITAAIGIA ITIGIAACIA GIACGCAAGI TTTICAAITA GAAAAGITGI CGACAGIAIT ICAACAGIGC CGGCICIGAA IAICAGCGAA ACAAAAAIAA AAAAITACAI AAACAITGAI CAIGCGIICA 301

AGATAACGAT GITTGCGCAT GCGACTATAG 401 TCACGTAAAA AGGGTATCTA GAATTATGAA GAAGAATATC GCATTTCTTC TTGCATCTAT GTTCGTTTTT TCTATTGCTA CAAACGCGTA CGCTGATATC A D I anti-VEGF Light Chain^ N A Y SIAT AGIGCATITI ICCCAIAGAI CITAAIACII CITCIIAIAG CGIAAAGAAG AACGIAGAIA CAAGCAAAAA ٧ ټ Œ, S Ø A F L L STII Signal TIR K N H X

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE

CTCCCTGTCC GCCTCTGTGG GCGATAGGGT CACCATCACC TGCAGGGAA GTCAGGATAT TAGCAACTAT TTAAACTGGT AATTTGACCA Z GIGGIAGIGG ACGICGCGIT CAGICCIAIA AICGIIGAIA S variable light (VL) cys ΙΩΟ CSAS H H Н CGGAGACACC CGCTATCCCA A S V G D R V GAGGGACAGG တ ,, ഗ 501 CAGTTGACCC AGTCCCCGAG TCAGGGGCTC S Д S GTCAACTGGG H J o 26

CAAGACCCTG TCTCTCCACT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC ტ S AGAGAGGTGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC GAAGTGGAGG CITCACCTCC F T S ACCAGGAAAA GCTCCGAAAG TACTGATTTA CGAGGCTTTC ATGACTAAAT r r > A P K TGGTCCTTTT დ ჯ ATCAACAGAA TAGTTGTCTT o, ø 601 9

CGGTCTTCTG AAGCGTTGAA TAATGACAGT TGTCATATCG TGGCACGGCA CCTGCAAACC TGTCCCATGG ტ ø GGACGTTTGG ATTACTGTCA ACAGTATAGC ACCGTGCCGT 3 ۷ ه S X O α υ × TTCGCAACTT × H FA GCCAGAAGAC Ω 闰 Д CGTCAGACGT GCAGTCTGCA ø ı ഗ GACTGGTAGT CTGACCATCA S H GGATTTCACT CCTAAAGTGA H Œ Ω 701 93

^Variable light cys

CGGTAGACTA CTCGTCAACT TTAGACCTTG ACGAAGACAAA CACACGACG
PSDEOLKSGTASVCLL GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT GTGTGCCTGC r L Q L K ы Ω S Д GGTAGACAGA AGTAGAAGGG CCATCTGTCT TCATCTTCCC Œ > S Q, TGTGGCTGCA AGITITGCITG ACACCGACGI æ 4 > AAGGTGGAGA TCAAACGAAC œ × TTCCACCTCT Œ > × 801 126

Constant Light cys^

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE

Attorney Name: KATHERINE M. KOWALCHYK

Phone No.: 612.371.531

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GTCACAGAGC AGGACAGCAA GCCCATTGAG GGTCCTCTCA CAGTGTCTCG TCCTGTCGTT GGCCTGAGC CCCGGACTCG CAAAGICTAC GCCTGCGAAG ICACCCATCA GGTGGATAAC GCCCTCCAAT CGGGTAACTC CCAGGAGAGT S ы ø z ט CGGGAGGTTA ACGAGAAACA o ы Ø AAAGCAGACT CCACCTATTG N O N GAGGCCAAAG TACAGTGGAA CICCGGITIC AIGICACCIT GACGCTGAGC W ø CCTGTCGTGG ATGTCGGAGT CGTCGTGGGA GCAGCACCCT A 臼 CTATCCCAGA TACAGCCTCA GATAGGGTCT ĸ 901 TGAATAACTT ACTTATTGAA GGACAGCACC NN

בי O CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG CGGACGCTTC AGTGGGTAGT T L S K A D Y E K H K V Y A C E V T H O ^Constant Light cys S တ 193

ACCCCGGACG CATCGTGGCG AGCTCGGTAC CCGGGGATCT AGGCCTAACG CTCACAATTA ATTTAGGAGA TGCGGCCTGC GTAGCACCGC TCGAGCCATG GGCCCCTAGA TCCGGATTGC start lambda t0 terminator' GAGTGTTAAT TAAATCCTCT cys to bind heavy 凶 CAACAGGGGA GITGICCCCT Ö æ z CAAAGAGCTT GTTTCTCGAA S TCGCCCGTCA AGCGGGCAGT Д 1101

GAGCCAACGG CGGCCCGCAA AAAATAACAA CGGCTGCGCG TAGAGCTTAC TTGACACACG CGTCCATCTT CGAAACCTCT AATAGCAGTG ACGTTACGAA CTOGGTIGCC GCCGGCGTI TITIAITGII GCCGACGCGC AICTCGAAIG AACTGIGIGC GCAGGIAGAA GCIITGGAGA ITAICGICAC IGCAAIGCII ^end lambda t0 terminator 1201

GCCCCTGTAC GAGGTAAAGC CCGATGCCAG CATTCCTGAC GACGATACGG GCTTGTCGCC AACTAACTAG TCCATCTCCC CCGCGACATG CTCCATTTCG GGCTACGGTC GTAAGGACTG CTGCTATGCC CCAACAGCGG TTGATTGATC AGGTAGAGGG CGCAAAATGA GCGTTATACC GCGTTTTACT CGCAATATGG 1301

AGCTGCTGCG CGATTACGTA AAGAAGTTAT TGAAGCATCC TCGTCAGTAA AAAGTTAATC TTTTCAACAG CTGTCATAAA GTTGTCACGG CCGAGACTTA ICGACGACGC GCTAATGCAT TICTICAATA ACTICGIAGG AGCAGICAIT TIICAATIAG AAAAGITGIC GACAGTAITI CAACAGIGCC GGCTCTGAAI 1401

GITITIAITI ITIAAIGIAI ITGIAACIAG IACGCAAGII CACGIAAAAA GGGIAICIAG AAITAIGAAG AAGAAIAICG CAITICITCI ATCAGCGAAA CAAAAATAAA AAATTACATA AACATTGATC ATGCGTTCAA GTGCATTTTT CCCATAGATC TTAATACTTC TTCTTATAGC MKKNIA TAGTCGCTTT 1501

\*STII Signal TIR-1

TTCAGTTAAA acgiagatac aagcaaaaaa gataacgaig titgcgcaig cgagiccaag icgaccacgi cagaccgcgi ciccactiti icggiccccg aagicaatiti ഗ ITCGITITIT CIAITGCTAC AAACGCGTAC GCTCAGGTTC AGCTGGTGCA GTCTGGCGCA GAGGTGAAAA AGCCAGGGGC E < A Q V Q L V Q S G A E V K
^anti-VEGF Heavy Chain (VNERK version) N A H 4 н S Œ > 1601 TGCATCTATG S K

Heavy Chain FR1 changed to SubgroupI consensus sequence

ACCTAATIGI GTAICCTGIA AAGCTICTGG CIATACCTIC ACCAACTAIG GIAIAAACTG GGICCGICAG GCCCCGGGIA AGGGCCTGGA AIGGGTIGGA TACCCAACCT ი > CCAGGCAGTC CGGGGCCCAT TCCCGGACCT ы O v Д o œ > TGGTTGATAC CATATTTGAC T N Y G I N W CATAGGACAT TTCGAAGACC GATATGGAAG YTF ^Variable Heavy (VH) cys Ö r R တ

GGATATGGCC ACTIGGCTGG ATACGACGCC TAAAGTTTGC AGCAAAGIGA AAAAGAAATC TGTGGAGGTT TTCGTGTCGT ATGGACGTCT ACTTGTCGGA TGAACAGCCT TACCTGCAGA TCGTTTCACT TTTTTTTAG ACACCTCCAA AAGCACAGCA ۲ ഗ × Н Д Ļ S ĹŁ, Œ, œ ATTTCAAACG × (z., TATGCTGCGG A A CCTATACCGG TGAACCGACC 77

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK

CCCAGTTCCT GGGTCAAGGA Ö CGCCTTCTCG GTGACCATAA AGCTGCAGAC TCGACGTCTG × GCGGAAGAGC CACTGGTATT X M R K S CCGCACTAIT AIGIGAACGA GGCGTGATAA TACACTTGCT P H Y Y V N E GCGCGCTGAG GACACTGCCG TCTATTACTG TGCAAAGTAC CTGTGACGGC AGATAATGAC ACGTTTCATG A K Y ^ин сув CGCGCGACTC K

TICCCGGGIA GCCAGAAGGG GGACCGIGGG AGGAGGTICI CGIGGAGACC CCCGIGICGC CGGGACCCGA Constant Heavy 1 (CH1) cys^ TCCTCCAAGA GCACCTCTGG GGGCACAGCG G T A s G ۲ S S N S GGCCTCCACC AAGGGCCCAT CGGTCTTCCC CCTGGCACCC Д L A Д Œ > S Д Ö TGGGACCAGT GGCAGAGGAG CCGGAGGTGG S ø CCGTCTCCTC တ Ŋ > 2001 ACCCTGGTCA Н H 143

GECECCTGA CCAGCGGCGT GCACACCTTC CCGGCTGTCC TACAGTCCTC GGCCGACAGG ATGTCAGGAG CCGCGGGACT GGTCGCCGCA CGTGTGGAAG E E Ħ ა ა S GALT GTGGAACTCA CACCTTGAGT တ z z 2101 GCCTGGTCAA GGACTACTTC CCCGAACCGG TGACGGTGTC CGGACCAGTT CCTGATGAAG GGGCTTGGCC ACTGCCACAG ഗ > PV Ш Д D Y F L V K

CAACACCAAG GTTGTGGTTC TCCCTCAGCA GCGTGGTGAC TGTGCCCTCT AGCAGCTTGG GCACCCAGAC CTACATCTGC AACGTGAATC ACAAGCCCAG TGTTCGGGTC ۵, × TCGTCGAACC CGTGGGTCTG GATGTAGACG TTGCACTTAG S S L G T Q T Y I C N V N H TCCTGAGATG AGGGAGTCGT CGCACCACTG ACACGGGAGA G L Y S L S S V V T V P S AGGACTCTAC

TGGCAGTCAG AAGGAGAAGG ACCGTCAGTC > S Д GIGGACAAGA AAGIIGAGCC CAAAICIIGI GACAAAACIC ACACAIGCCC ACGIGCCCA GCACCIGAAC ICCIGGGGG hinge cys CICAIGAICI CCCGGACCCC IGAGGICACA IGCGIGGIGG IGGACGIGAG CCACGAAGAC CCIGAGGICA AGIICAACIG GGACTCCAGT > Д GAGTACTAGA GGGCCTGGGG ACTCCAGTGT ACGCACCACC ACCTGCACTC GGTGCTTCTG Ω H ^Constant Heavy 2 (CH2) cys S V O CVVV EVT Ф Ξ GTTCCTGTGG CAAGGACACC Ω × 2401 CCCCAAAACC GGGGTTTTGG × ۵

2501 GTACGTGGAC GCCGTGGAGG TGCATAATGC CAAGACAAAG CCGCGGGAGG AGCAGTACAA CAGCACGTAC CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC CGCAGGAGTG CCGCACCTCC ACGTATTACG GTTCTGTTTC GGCGCCCTCC TCGTCATGTT GTCGTGCATG GCACACCAGT G V E V V S Y N S T Y R V V S CATGCACCTG 310 Y D

CCCGTCGGGG CAAAGCCAAA GGGCAGCCCC GTTTCGGTTT K × GGTTGTTTCG GGAGGGTCGG GGGTAGCTCT TTTGGTAGAG N K A L P A P I E K T I S TGAATGGCAA GGAGTACAAG TGCAAGGTCT CCAACAAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC CCTCATGTTC ACGTTCCAGA E Y K C K V S GICCIGACCG ACTIACCGIT ט 2601 CAGGACTGGC Δ GATGACCAAG AACCAGGTCA GCCTGACCTG CCTGGTCAAA GGCTTCTATC CCAGCGACAT TIGGICCAGI CGGACTGGAC GGACCAGITT CCGAAGAIAG GGICGCIGIA GFYPS L L ß > 0 CTACTGGTTC M T X GGTGTACACC CTGCCCCCAT CCCGGGAAGA GACGGGGTA GGGCCCTTCT L P P S R E E CCACATGTGG V Y T 2701 GAGAACCACA CTCTTGGTGT ы 377

\*Constant Heavy 3 (CH3) cys

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK

start of tet resistance promoter

^-35 of promoter

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GTCGTTCGAG GAGAGGGACA CTCCGGGTAA ATAAGCATGC GACGGCCCTA GAGTCCCTAA CGCTCGGTTG CCGCCGGGCG TTTTTTATTG TTAACTCATG TTTGAGAGCT TATCATCGAT GAGGCCCATT TATTCGTACG CTGCCGGGAT CTCAGGGATT GCGAGCCAAC GGCGGCCCGC AAAAAATAAC AATTGAGTAC AAACTGTCGA ATAGTAGCTA TCTTCCTCTA CAGCAAGCTC CTCTCCCTGT S × CTGCCGAGGA AGAAGGAGAT ^end lambda to terminator GCAGAAGAGC CGTCTTCTCG S × o GGAGAACAAC TACAAGACCA CGCCTCCCGT GCTGGACTCC GACGGCTCCT ACCACTACAC TGGTGATGTG S × Ö CCTCTTGTTG ATGITCTGGT GCGGAGGGCA CGACCTGAGG GCTCTGCACA CGAGACGTGT LHI ഗ K 'start lambda to terminator CATGCTCCGT GATGCATGAG TTGCAGAAGA GTACGAGGCA CTACGTACTC ы Ξ c s v E Y AACGTCTTCT z N V F z 凶 2801 CGCCGTGGAG TGGGAGAGCA ATGGGCAGCC TACCCGTCGG GCAGCAGGGG TGGCACCIGI TCTCGTCCAC CGTCGTCCCC а Ö o ø ტ ø GCGCCACCTC ACCCTCTCGT AGAGCAGGTG 3 2 凶 ഗ CTCCGGGTAA 2901 ACCGTGGACA A V E Ö Д ۲ 410 443 477

TICGADATTÀ COCCAICADA INGIGICADI ITANCGAITG COTCAGICCO IGGCACAINC ITINGAITGI INCOCCGAGIN GCAGINGGAG CCGIGGCAGI CCCTGGATGC TGTAGGCATA GGCTTGGTTA TGCCGGTACT GCCGGGCCTC TTGCGGGATA TCGTCCATTC CGACAGCATC GCCAGTCACT ATGGCGTGCT GGGACCTACG ACATCCGTAT CCGAACCAAT ACGGCCATGA CGGCCCGGAG AACGCCCTAT AGCAGGTAAG GCTGTCGTAG CGGTCAGTGA TACCGCACGA 'start of tet resistance translation ^-10 region of tet resistance promoter 3201

3101 AAGCITIAAI GCGGIAGIII AICACAGIIA AAITGCIAAC GCAGICAGGC ACCGIGIAIG AAAICIAACA AIGCGCICAI CGICAICCIC GGCACCGICA

3301

# **IG. 18A**

GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAAATC TCATTGCTGA GTTGTTATTT AAGCTTGCCC AAAAAGAAGA AGAGTCGAAT TITITICITCI TCTCAGCITA TTCGAACGGG CTTAAGTIGA AGAGGIAIGA AACCTAITICC ITTAIGICIG IACITITIAG AGIAACGACT CAACAATAAA GTTGATTGAT CAGGTAGAGG CTTGACACAC GCGTCCATCT TCGAAACCTC TAATAGCAGT GACGTTACGA AGCGTTATAC CGCGTTTTAC TGGTTGTCGC CAACTAACTA GTCCATCTCC AGCITITGGAG AITAICGICA CIGCAAIGCI ICGCAAIAIG GCGCAAAAIG ACCAACAGCG CGCAGGTAGA GAACTGTGTG

GCATTCCTGA CGACGATACG GAGCTGCTGC GCGATTACGT AAAGAAGTTA TTGAAGCATC CTCGTCAGTA CCCGCGACAT GCTCCATTIC GGGCTACGGT CGIAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TITCTICAAT AACTTCGTAG GAGCAGTCAT CCCGATGCCA CGAGGTAAAG GGGCGCTGTA 201

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> TTTGTAACTA GTACGCAAGT TITICAATIA GAAAAGIIGI CGACAGIAIT ICAACAGIGC CGGCICIGAA IAICAGCGAA ACAAAAAIAA AAAAIIACAI AAACAIIGAI CAIGCGIICA CITITICAACA GCIGICAIAA AGIIGICACG GCCGAGACII AIAGICGCII IGIITITIAI ITIITAAIGIA AAAAGTTAAT 301

TCACGTAAAA AGGGTATCTA GAATTATGAA GAAGAATATC GCATTTCTTC TTGCATCTAT GTTGGTTTTT TCTATTGCTA CAACGGTA CGCTGATATC AGTGCATTTT TCCCATAGAT CTTAATACTT CTTCTTATAG CGTAAAGAAG AACGTAGATA CAAGCAAAAA AGATAAACGAT GTTTGCGCAT GCGACTATAG anti-VEGF Light Chain SIATNAY F V F A S M ASTII Signal TIR -1 401

GCCTCTGTGG GCGATAGGGT CACCATCACC TGCAGCGCAA GTCAGGATAT TAGCAACTAT TTAAACTGGT CGGAGACACC CGCTATCCCA GTGGTAGTGG ACGTCGCGTT CAGTCCTATA ATCGTTGATA AATTTGACCA ^variable light (VL) cys C S A S T I Ω > S CICCCIGICC GAGGGACAGG ഗ ы Ø CAGITICACCC AGICCCCGAG TCAGGGGCTC S ഗ GTCAACTGGG 501 56

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK

TCTCTCCACT CTGGAGTCCC TTCTCGCTTC TCTGGATCCG GTTCTGGGAC AGAGAGGTGA GACCTCAGGG AAGAGCGAAG AGACCTAGGC CAAGACCCTG S G r SRF G V P SLHS ACCAGGAAAA GCTCCGAAAG TACTGATTTA CTTCACCTCC CGAGGCTTTC ATGACTAAAT GAAGTGGAGG F T S r ı x APKV TGGTCCTTT ע צ Д 501 ATCAACAGAA TAGTTGTCTT о о 9

GCCAGAAGAC TICGCAACTI AITACIGICA ACAGIATAGC ACCGIGCCGI GGACGTITGG ACAGGGIACC CGGICTICIG AAGCGIIGAA IAAIGACAGI IGICAIAIGG IGGCACGGCA CCIGCAAACC IGICCAIGG P E D F A I Y Y C Q Q Y S I V P W I F G Q G I ^Variable light cys GCAGTCTGCA GACTGGTAGT CGTCAGACGT S CTGACCATCA E GGATTTCACT CCTAAAGTGA (L) Δ 701

TICCACCICI AGITIGCITG ACACCGACGI GGIAGACAGA AGIAGAAGGG CGGIAGACTA CICGICAACI TIAGACCITG ACGAAGACAA CACACGGACG K V E I K R I V A A P S V F I F P P S D E Q L K S G I A S V V C L L TOTGGCTGCA CCATCTUTCT TCATCTTCCC GCCATCTGAT GAGCAGTTGA AATCTGGAAC TGCTTCTGTT GTGTGCCTGC Constant Light cys^ S K > TCAAACGAAC × 801 AAGGTGGAGA > × 126

\*Constant Light cys

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK Phone No.: 612.371.5311

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GTCACAGAGC AGGACAGCAA TCCTGTCGTT GCCTGCGAAG TCACCCATCA GGGCCTGAGC CCCGGACTCG GTTTCAGATG CGGACGCTTC AGTGGGTAGT CAGTGTCTCG ы H > GGTGGATAAC GCCCTCCAAT CGGGTAACTC CCAGGAGAGT CEV GGTCCTCTCA ß 囟 o GCCCATTGAG CAAAGTCTAC Α Α **z** × ACGAGAAACA TITCGICTGA TGCTCTTTGT CGGGAGGTTA ഗ ø × A L 凶 AAAGCAGACT CCACCTATTG × V D N Ω K A CTATCCCAGA GAGGCCAAAG TACAGTGGAA CTGCGACTCG GACGCTGAGC ATGTCACCTT 3 ഗ н ø H CTCCGGTTTC EAKV CGTCGTGGGA GCAGCACCCT ы H S ATGTCGGAGT GATAGGGTCT TACAGCCTCA Y P R ы S TGAATAACTT ACTTATTGAA CCTGTCGTGG GGACAGCACC N N F တ Ω GAGTETTAAT TAAATCCTCT ACGCCGGACG CATCGTGGCG AGCTCGGTAC CCGGGGATCT AGGCCTAACG CTCACAATTA ATTTAGGAGA TGCGGCCTGC GTAGCACCGC TCGAGCCATG GGCCCCTAGA TCCGGATTGC start lambda to terminator^ cys to bind heavy 0 ы GTTGTCCCCT CAACAGGGGA Ö œ z CAAAGAGCTT GTTTCTCGAA Ŀ ß × TCGCCCGTCA AGCGGGCAGT > S 1101

GAGCCAACGG CGGCCCGCAA AAAATAACAA CGGCTGCGCG TAGAGCTTAC TTGACACACG CGTCCATCTT CGAAACCTCT AATAGCAGTG ACGTTACGAA TGCAATGCTT GCTTTGGAGA TTATCGTCAC GCCGACGCGC ATCTCGAATG AACTGTGTGC GCAGGTAGAA ^end lambda to terminator GCCGGGCGTT TITTATTGTT CTCGGTTGCC 1201

TIGATIGATO AGGIAGAGGG GGCGCTGTAC GAGGIAAAGC CCGAIGCCAG CATICCIGAC GACGAIACGG GCGTTATACC GCGTTTTACT GGTTGTCGCC AACTAACTAG TCCATCTCCC CCGCGACATG CTCCATTTCG GGCTACGGTC GTAAGGACTG CTGCTATGCC CCAACAGCGG CGCAAAATGA CGCAATATGG

CCGAGACTTA TCGACGACGC GCTAATGCAT TICTICAATA ACTICGIAGG AGCAGICATI TITCAATTAG AAAAGITGIC GACAGTAITI CAACAGTGCC GGCTCTGAAT AGCIGCIGCG CGAITACGIA AAGAAGITAI IGAAGCAICC ICGICAGIAA AAAGITAAIC ITITCAACAG CIGICAIAAA GITGICACGG 1401

CATTICITCI TAGTCGCTTT GITTITIAITT TITAAIGIAI TIGIAACIAG IACGCAAGIT CACGȚAAAAA GGGIAICIAG AAITAIGAAG AAGAAIAITCG AICAGCGAAA CAAAAAIAAA AAAITACAIA AACAITGAIC AIGCGIICAA GIGCAITITI CCCAIAGAIC ITAAIACIIC IICITAIAGC KNIA STII Signal TIR-1 × 1501 TAGTCGCTTT

CTGAGAGAGG CIAITGCIAC AAACGCGIAC GCTCAGGIIC AGCIGCAAGA GICIGGCCCG GGCCIGGIGA AACCAICICA GACICICCC 'Heavy chain FRI changed to Subgroup II consensus sequence ACGIAGAIAC AAGCAAAAAA GAIAACGATG TITGCGCATG CGAGICCAAG ICGACGITCI CAGACCGGGC CCGGACCACI ITGGIAGAGI ഗ Д G L V K ^anti-VEGF heavy chain (VNERK version) S G P 3 0 1 0 v 0 K > K z Н ø 1601 TGCATCTATG TTCGTTTTTT Ĺ > Σ ഗ Þ

ACCTAATIGI CATAITIGAC CCAGGCAGIC CGGGCCCAI ICCCGGACCI TACCCAACCI ATGGGTTGGA > GTATAAACTG GGTCCGTCAG GCCCCGGGTA AGGGCCTGGA v ტ Д o 2 > N GATATGGAAG TGGTTGATAC Y T F T N Y G CTATACCTTC ACCAACTATG YT ^ T V S G Y T F ^Variable Heavy (VH) cys 1701 INGACINGIA CIGITICIGG AACTGAACAT GACAAAGACC H 43

TGAACAGCCT TTCGTGTCGT ATGGACGTCT ACTTGTCGGA TCGTTTCACT TTTTCTTTAG ACACCTCCAA AAGCACAGCA TACCTGCAGA ഗ TGTGGAGGTT ഗ H AGCAAAGTGA AAAAGAAATC Ω H ഗ H Œ, œ TATGCTGCGG ATTTCAAACG ATACGACGCC TAAAGTTTGC × Ŀ Ω Þ TGAACCGACC GGATATGGCC ACTTGGCTGG H Δ, CCTATACCGG H 1801 77

Docket No.: 11669.120USUI
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK

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CGCCTTCTCG GTGACCATAA AGCTGCAGAC CCCAGTTCCT GCGGAAGAGC CACTGGTATT TCGACGTCTG GGGTCAAGGA Ö × 3 ഗ × ď GCGCGCTGAG GACACTGCCG TCTATTACTG TGCAAAGTAC CCGCACTATT ATGTGAACGA CIGIGACGGC AGAIAAIGAC ACGITITCAIG GGCGIGAIAA IACACIIGCI z н × A ^ин сув > × 4 H CGCGCGACTC 囟 4 ద

GGACCGTGGG AGGAGGTTCT CGTGGAGACC CCCGTGTCGC CGGGACCCGA GGCCTCCACC AAGGGCCCAT CGGTCTTCCC CCTGGCACCC TCCTCCAAGA GCACCTCTGG GGGCACAGCG GCCCTGGGCT Constant Heavy 1 (CH1) cys^ G T A ა დ H S × S S Д K н CCGGAGGTGG ITCCCGGGTA GCCAGAAGGG Д [z, > S Д Ö × H S 4 2001 ACCCTGGTCA CCGTCTCCTC TGGGACCAGT GGCAGAGGAG S ß > H H 143

GGCCGACAGG ATGTCAGGAG ø CCGGCTGTCC P A GIGGAACTCA GGCGCCCTGA CCAGCGGCGT GCACACCTTC CCGCGGGACT GGTCGCCGCA CGTGTGGAAG H F Ξ ک ن S ۲ ы d D CCTGATGAAG GGGCTTGGCC ACTGCCACAG CACCTTGAGT ഗ Z Z GGACTACTIC CCCGAACCGG IGACGGIGIC ß > ۲ > Ճ M Д ۲4 ج Ω 2101 GCCTGGTCAA CGGACCAGTT LVK

CAACACCAAG z GCGTGGTGAC TGTGCCCTCT AGCAGCTTGG GCACCCAGAC CTACATCTGC AACGTGAATC ACAAGCCCAG TGTTCGGGTC Д TCGTCGAACC CGTGGGTCTG GATGTAGACG TTGCACTTAG Y I C N V N H ^CH1 cys H ø ۲ r G ഗ တ TCCTGAGATG AGGGAGTCGT CGCACCACTG ACACGGGAGA G L Y S L S S V V T V P S TCCCTCAGCA 2201 AGGACTCTAC

TGGCAGTCAG AAGGAGAAGG
PSVFLFP TTCCTCTTCC ACCGTCAGTC CACCTGITCT TYCAACTCGG GITIAGAACA CTGITITYGAG TGTGTACGGG TGGCACGGGI CGTGGACTTG AGGACCCCCC
V D K K V E P K S C D K T H T C P P C P A P E L L G G
^cys to bind light chain ^hinge cys AAGTTGAGCC CAAATCTTGT GACAAAACTC ACACATGCCC ACCGTGCCCA GCACCTGAAC TCCTGGGGG hinge cys GTGGACAAGA

CAAGGACACC CICAIGAICI CCCGGACCCC IGAGGICACA IGCGIGGIGG IGGACGIGA CCACGAAGAC CCIGAGGICA AGIICAACIG TCAAGTTGAC V K GITCCIGIGG GAGIACIAGA GGGCCIGGGG ACTCCAGIGI ACGCACCACC ACCIGCACTC GGIGCITCIG GGACICCAGI 囟 Δ H ^Constant Heavy 2 (CH2) cys S V Q CVV ۲ ۲ ы Д H α S H Ω × 2401 CCCCAAAACC GGGTTTTGG

CCGCGGGGGG AGCAGTACAA CAGCACGTAC CGTGTGGTCA GCGTCCTCAC CGTCCTGCAC GCAGGACGTG CGCAGGAGTG L GGCGCCCTCC TCGTCATGTT GTCGTGCATG GCACACCAGT v v æ STX z × 闰 ы В GGCGTGGAGG TGCATAATGC CAAGACAAAG CCGCACCTCC ACGTATTACG GITCTGITTC FI X × H N A > Œ ა ა 2501 GTACGTGGAC CATGCACCTG >

CCCGTCGGGG GGCAGCCCC CAAAGCCAAA GTTTCGGTTT × Ø × GGGTAGCTCT TTTGGTAGAG P I E K T I S CCAACAAGC CCTCCCAGCC CCCATCGAGA AAACCATCTC GGITGITICG GGAGGGICGG N K A L P A TGAATGGCAA GGAGTACAAG TGCAAGGTCT ACGITCCAGA CKVS CCTCATGITC X X Θ ACTTACCGTT × Ö z CAGGACTGGC GTCCTGACCG Ω o

AACCAGGICA GCCIGACCIG CCIGGICAAA GGCITCIAIC CCAGCGACAI INGGICCAGI CGGACIGGAC GGACCAGITI CCGAAGAIAG GGICGCIGIA CLVKGFYPS \*Constant Heavy 3 (CH3) cys L T S > œ z GATGACCAAG CTACTGGTTC × Ξ GGTGTACACC CTGCCCCCAT CCCGGGAAGA CCACATGTGG GACGGGGTA GGGCCCTTCT ω ы ~ ഗ а ۵, ч × > CTCTTGGTGT 2701 GAGAACCACA ø Δ, 377

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE

Attorney Name: KATHERINE M. KOWALCHYK

Phone No.: 612.371.5311

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GTCGTTCGAG CGTCTTCTCG GAGAGGGACA CAGCAAGCTC CTCTCCCTGT × TCTTCCTCTA AGAAGGAGAT GCAGAAGAGC S ø TGGTGATGTG GCTGGACTCC GACGGCTCCT CTGCCGAGGA GCTCTGCACA ACCACTACAC ß × ט Ξ CGAGACGTGT CGACCTGAGG z S Q H ч K GGAGAACAAC TACAAGACCA CGCCTCCCGT CCTCTTGTTG ATGTTCTGGT GCGGAGGGCA AACGICITCI CATGCICCGI GATGCATGAG TTGCAGAAGA GTACGAGGCA CTACGTACTC РР ш Ξ c s v ^снз сув YKT z N V z 2801 CGCCGTGGAG TGGGAGGCA ATGGGCAGCC GCGGCACCTC ACCCTCTCGT TACCCGTCGG GCAGCAGGGG CGTCGTCCCC Q Q <sub>ເ</sub> Ö œ AGAGCAGGTG TGGCACCTGT TCTCGTCCAC 3 တ œ S 2901 ACCGTGGACA Ω > 4 ۲ 443

3001 CTCCGGGTAA ATAAGCATGC GACGGCCCTA GAGTCCCTAA CGCTCGGTTG CCGCCGGGCG TTTTTTATTG TTAACTCATG TITTGACAGCT TATCATCGAT GAGGCCCATT TATTCGTACG AAAGTAGCAA ATAGTAGCTA ATAGTAGCTA 0 P G K 477

start lambda to terminator cend lambda to terminator

3101 AAGCITTAAT GCGGTAGITT ATCACAGITA AATTGCTAAC GCAGTCAGGC ACCGTGTATG AAATCTAACA ATGCGCTCAT CGTCATCCTC GGCACCGTCA TTCGAAATTA CGCCATCAAA TAGTGTCAAT TTAACGATTG CGTCAGTCG TGGCACATAC TTTAGATTG TACGCGAGTA GCAGTAGGAG CCGTGGCAGT \*start of tet resistance translation ^-10 region of tet resistance promoter

CCCIGGAIGC IGIAGGCAIA GGCITGGITA IGCCGGIACI GCCGGGCCIC IIGCGGGAIA ICGICCAIIC CGACAGCAIC GCCAGICACI AIGGCGIGCI GGGACCTACG ACATCCGTAT CCGAACCAAT ACGGCCATGA CGGCCCGGAG AACGCCCTAT AGCAGGTAAG GCTGTCGTAG CGGTCAGTGA TACCGCACGA 3201

3301

Docket No.: 11669.120USU1 Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK

Phone No.: 612.371.5311

Inventor: SIMMONS

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AGAGTCGAAT CTTAAGTTGA AGAGGTATGA AACCTATTCC TTTATGTCTG TACTTTTTAG AGTAACGACT CAACAATAAA TTCGAACGGG TTTTTCTTCT TCTCAGCTTA AAAAAGAAGA AAGCTTGCCC GTTGTTATTT TCATTGCTGA ATGAAAATC AAATACAGAC TTGGATAAGG TCTCCATACT GAATTCAACT

CAGGTAGAGG GTCCATCTCC GTTGATTGAT CAACTAACTA AGCTTTGGAG ATTATCGTCA CTGCAATGCT TCGCAATATG GCGCAAAATG ACCAACAGCG TCGAAACCTC TAATAGCAGT GACGTTACGA AGCGTTATAC CGCGTTTTAC TGGTTGTCGC GCGCAAAATG ACCAACAGCG CTTGACACAC GCGTCCATCT CGCAGGTAGA GAACTGTGTG 101

CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TTTCTTCAAT AACTTCGTAG GAGCAGTCAT CTCGTCAGTA TTGAAGCATC AAAGAAGTTA GAGCTGCTGC GCGATTACGT CGACGATACG GCATTCCTGA GGGCTACGGT CCCGATGCCA CGAGGTAAAG GCTCCATTTC GGCCCTGTA CCCGCGACAT 201

GTACGCAAGT TITICAAITA GAAAAGITGI CGACAGIAIT ICAACAGIGC CGGCICIGAA IAICAGCGAA ACAAAAITAA AAAAITACAI AAACAIIGAI CAIGCGITCA TTTGTAACTA TTTTAATGTA GCTGTCATAA AGTIGTCACG GCCGAGACTT ATAGTCGCTT TGTTTTTATT CTTTTCAACA AAAAGTTAAT 301

TCACGTAAAA AGGGTATCTA GAATTATGAA GAAGAATATC GCATTTCTTC TTGCATCTAT GTTCGTTTTT TCTATTGCTA CAAACGCGTA CGCTGATATC AACGTAGATA CAAGCAAAAA AGATAACGAT GTTTGCGCAT GCGACTATAG Anti-VEGF Light chain (version Y0317)^ S Ø CGTAAAGAAG AFLL CTTAATACTT CTTCTTATAG Signal TIR X X AGTGCATTTT TCCCATAGAT 401

AATTTGACCA TGCAGCGCAA GTCAGGATAT TAGCAACTAT TTAAACTGGT z ATCGTTGATA S CAGTCCTATA Ω ø တ ACGTCGCGTT S CACCATCACC GTGGTAGTGG ۲ н H CICCCIGIC GCCICIGIGG GCGAIAGGGI CGCTATCCCA œ Ω CGGAGACACC Ö > A S GAGGGACAGG ഗ H S CAGITGACCC AGICCCCGAG TCAGGGGCTC Д GTCAACTGGG H ч o 501

CAAGACCCTG GTTCTGGGAC v TCTGGATCCG AGACCTAGGC Ö Ö TICICGCITC AAGAGCGAAG Ĺ ĸ တ GACCTCAGGG CTGGAGTCCC > Ö AGAGAGGTGA TCTCTCCACT Ø H တ GAAGTGGAGG CTTCACCTCC ß H 124 TACTGATTTA ATGACTAAAT н CGAGGCTTTC GCTCCGAAAG × A P ACCAGGAAAA TGGTCCTTTT × U а ATCAACAGAA TAGTTGTCTT ο × ø 601 9

TGTCCCATGG ACAGGGTACC ט GGACGTTTGG CCTGCAAACC Ŀ Н ACCGTGCCGT TGGCACGGCA 4 > £ ACAGTATAGC TGTCATATCG S × O ATTACTGTCA TAATGACAGT O ບ × TTCGCAACTT AAGCGTTGAA FAT GCCAGAAGAC CGGTCTTCTG Ω E Д GCAGTCTGCA CGTCAGACGT Ø S CTGACCATCA GACTGGTAGT CCTAAAGTGA GGATTTCACT (z, Ω 701 93

CACACGGACG GTGTGCCTGC ں > TGCTTCTGTT ACGAAGACAA ഗ K AATCTGGAAC TTAGACCTTG Ö ß GAGCAGTTGA CTCGTCAACT × ы ø ы GCCATCTGAT CGGTAGACTA Ω S Д TCATCTTCCC AGTAGAAGGG [t<sub>4</sub> GGTAGACAGA P S V F CCATCTGTCT TGTGGCTGCA ACACCGACGT 4 4 > TCAAACGAAC AGTITIGCTIG œ AAGGTGGAGA TTCCACCTCT ы 801

GTCACAGAGC · AGGACAGCAA S CAGTGTCTCG > CCAGGAGAGT GGTCCTCTCA S ω o CGGGTAACTC GCCCATTGAG S z ტ GCCCTCCAAT CCACCTATTG CGGGAGGTTA Ø ы GGTGGATAAC z Δ > TACAGTGGAA CTCCGGTTTC ATGTCACCTT ø GAGGCCAAAG > 4 CTATCCCAGA GATAGGGTCT œ TGAATAACTT ACTTATTGAA z 106

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GCAGCACCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC GCCTGCGAAG TCACCCATCA GGGCCTGAGC	T CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTTGT GTTTCAGATG CGGACGCTTC AGTGGGTAGT CCCGGACTCG	S
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ACA	TGI	Ħ
GAA	CIT	×
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CTCACAATTA ATTTAGGAGA TGCGGCCTGC GTAGCACCGC TCGAGCCATG GGCCCCTAGA TCCGGATTGC CCGGGGATCT AGGCCTAACG GAGTGTTAAT TAAATCCTCT ACGCCGGACG CATCGTGGCG AGCTCGGTAC 0 υ CAACAGGGGA GITGICCCT Ö œ z CAAAGAGCTT GTTTCTCGAA [z, တ × 1101 TCGCCCGTCA AGCGGGCAGT > Ω, ഗ

BAGCCAACGG CGGCCGCAA AAAATAACAA CGGCTGCGCG TAGAGCTTAC TTGACACACG CGTCCATCTT CGAAACCTCT AATAGCAGTG ACGTTACGAA TTATCGTCAC TGCAATGCTT GCCGACGCGC ATCTCGAATG AACTGTGTGC GCAGGTAGAA GCTTTGGAGA TITIATIGIT GCCGGGCGTT CICGGIIGCC 1201

GACGATACGG SCGITATACC GCGITITACI GGIIGICGCC AACIAACIAG ICCAICICCC CCGCGACAIG CICCAITICG GGCIACGGIC GIAAGGACIG CIGCIAIGCC CATTCCTGAC INGAITGAIC AGGIAGAGGG GCCCTGIAC GAGGIAAAGC CCGAIGCCAG CCAACAGCGG CGCAAAATGA CGCAATATGG 1301

GCIAAIGCAI IICIICAAIA ACIICGIAGG AGCAGICAII IIICAAIIAG AAAAGIIGIC GACAGIAIII CAACAGIGCC GGCICIGAAI CCGAGACTTA GITGICACGG CGAITTACGTA AAGAAGTTAI TGAAGCAICC ICGICAGIAA AAAGTIAAIC ITITICAACAG CIGICAIAAA AGCTGCTGCG TCGACGACGC 1401

TAGTCGCTTT GTTTTTATTT TTTAATGTAT TTGTAACTAG TACGCAAGTT CACGTAAAAA GGGTATCTAG AATTATGAAG AAGAATATCG CATTTCTTCT ATCAGCGAAA CAAAAATAAA AAATTACATA AACATTGATC ATGCGTTCAA GTGCATTTTT CCCATAGATC TTAATACTTC TTCTTATAGC GTAAAGAAGA TAGTCGCTTT 1501

STII Signal TIR-1

AAACGCGTAC GCTCAGGTTC AGCTGGTGCA GTCTGGCGCA GAGGTGAAAA AGCCAGGGGC TTCAGTTAAA TCGGTCCCCG AAGTCAATTT > v EVKK TITICCCCATG CGAGTCCAAG TCGACCACGT CAGACCGCGT CTCCACTITIT SGA r v o > Ø × K z CTATTGCTAC AAGCAAAAA GATAACGATG H 4 н TICCLITITI Ĺ, TGCATCTATG ACGTAGATAC Σ ഗ 4 1601

\*Heavy Chain FR1 changed to Subgroup1 consensus sequence

^Anti-VEGF (version Y0317) Heavy Chain

ATGGGTTGGA TGGATTAACA ACCTAATTGT TACCCAACCT GTATGAACTG GGTCCGTCAG GCCCCGGGTA AGGGCCTGGA TCCCGGACCT ტ CGGGGCCCAT × ტ Д 4 CCAGGCAGTC ø æ > CATACTTGAC M N M ACGCACTACG TGCGTGATGC × H H CTACGACTTC GATGCTGAAG [I4 Ω × AAGCITICIGG TTCGAAGACC Ö S 4 GTATCCTGTA CATAGGACAT U 1701

ATGGACGTCT ACTTGTCGGA TGAACAGCCT TACCTGCAGA o , TTCGTGTCGT AAGCACAGCA ø Н S AAAAGAAATC TGTGGAGGTT TITICITIAG ACACCICCAA × တ H Ω S نتا TANAGITITGC AGCAAAGIGA TCGTTTCACT H (z, æ ATTTCAAACG DZ, × 124 ATACGACGCC TATGCTGCGG Ø K ACTTGGCTGG TGAACCGACC H ۵, W GGATATGGCC CCTATACCGG × 1801

TTGGGACCAG AACCCTGGTC GGGGTCAAGG CCCCAGITCC o v TTCGACGTCT GGTGACCATA AAGCTGCAGA M V Q CCGTACTATT ATGGGACGAG CCACTGGTAT н м х TACCCTGCTC E E GGCATGATAA × P Y Y ACGITICATG TGCAAAGTAC A K Y AGATAATGAC TCTATTACTG X X CTGTGACGGC GACACTGCCG DTAV GCGCGCTGAG CGCGCGACTC R A E 1901

ACGGACCAGT TGCCTGGTCA CCGGGACCCG Ö ы 4 CCCCGTGTCG GGGCCACAGC T A Ö AGCACCTCTG TCGTGGAGAC Ö S ۲ S CTCCTCCAAG GAGGAGGTTC × S ഗ CCCTGGCACC GGGACCGTGG а ø ы AGCCAGAAGG TCGGTCTTCC Œ > S CAAGGGCCCCA GTTCCCGGGT Ö × CGGCCTCCAC GCCGGAGGTG ഗ TGGCAGAGGA S ACCGTCTCCT ഗ > H 143

Docket No.: 11669.120USUI
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 33 of 50

TTA X	AG	AC TG	g i o	ဗ္ဗ ႘	ညီ ညီ ဇ	E CT B	AC TG	AT X	<b>\$</b> E
SACTC STGAG L	GGTGGACAAG CCACCTGTTC V D K	CCCCCAAAAC GGGGGTTTTG P P K P	GGTACGTGGA CCATGCACCT Y V D	CCAGGACTGG GGTCCTGACC Q D W	CGAGAACCAC GCTCTTGGTG R E P Q	CGTG CACAC	CACCGTGGAC GTGGCACCTG T V D	TCTCCGGGTA AGAGGCCCAT S P G K	SCTTT
CAGG GTCC G			GGTP CCAT			TCGC AGCG			TAAC
STCCT	CCAA GGTT	CTTC	AACT STTGA N W	CTGCA GACGT L H	16000 10066 P	GACA SCTGT D I	AAGCT FTCGA K L	CCTG SGGAC L	ATCGA FAGCT
CTACAGTCCT CAGGACTCTA GATGTCAGGA GTCCTGAGAT L Q S S G L Y	GCAACACCAA CGTTGTGGTT N T K	CTTCCTCTTC GAAGGAGAAG F L F	AAGTTCAACT GGTACGTGGA TTCAAGTTGA CCATGCACCT K F N W Y V D	AGCGTCCTCA CCGTCCTGCA TCGCAGGAGT GGCAGGACGT S V L T V L H	AGGCAGCCC TCCCGTCGGG G Q P	CCCAGCGACA TCGCCGTGGA GGGTCGCTGT AGCGGCACCT P S D I A V E	TTCTTCCTCT ACAGCAAGCT AAGAAGGAGA TGTCGTTCGA F F L Y S K L	CCTCTCCCTG GGAGAGGGAC L S L	TTATCATCGA TAAGCTTTAA AATAGTAGCT ATTCGAAATT
GTC CCAG G		AGT O TCA G		CCTCA C SGAGT G			TCT AAGA 1		
CCCGGCTGTC GGGCCGACAG P A V	CACAAGCCCA GTGTTCGGGT H K P S	CCGTC GGCAG	CCCTGAGGTC GGGACTCCAG P E V	GTCC CAGG	CCAAAGCCAA GGTTTCGGTT K A K	AGGCTTCTAT TCCGAAGATA G F Y	TTCCI	CGCAGAAGAG GCGTCTTCTC Q K S	GTTTGACAGC CAAACTGTCG
		GA CTO		S AGCGT TCGCA S V	T CCA S GGI	4 AG0		44 CGC 17 GCC	
ACCT. STGGA/ T F	TGAA: ACTT!	ນ ນ ນນນນ ນອນນອ	GAAG! SCTTC1 E D	TGTGGTC ACACCAC V V	CATC GTAG	GGTCA CCAGT	GCTCC	CTAC GATG	CTCA
AGGCGCCCTG ACCAGCGGCG TGCACACCTT TCCGCGGGAC TGGTCGCCGC ACGTGTGGAA G A L T S G V H T F	CAACGTGAAT GTTGCACTTA N V N	CTCCTGGGGG GACCGTCAGT GAGGACCCCC CTGGCAGTCA L L G G P S V	GCCACGAAGA CGGTGCTTCT H E D	CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA GGCACACCAG TCGCAGGAGT GGCAGGACGT R V V S V L T V L H	TTTTGGTAGA GGTTTCGGTT K T I S K A K	GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CTTGGTCCAG TCGGACTGGA CGGACCAGTT TCCGAAGATA N Q V S L T C L V K G F Y	CGACGCTCC GCTGCCGAGG D G S	AACCACTACA CGCAGAAGAG TTGGTGATGT GCGTCTTCTC N H Y T Q K S	GTTAACTCAT CAATTGAGTA
A 5 55555		GAA ACTT E	GTGA SCACT V S			SACCT STGGA T C			
CAGC GTCG( S (	CCTACATCTG GGATGTAGAC Y I C	AGCACCTGAA TCGTGGACTT A P E	GTGGACGTGA CACCTGCACT V D V S	CCCCCATACA ACAGCACGTA CTCCTCATGT TGTCCTCCAT E Q Y N S T Y	CCCCATCGAG GGGGTAGCTC P I E	CCTG	ACGCCTCCCG TGCTGGACTC TGCGGAGGC ACGACCTGAG T P P V L D S	GGCTCTGCAC CCGAGACGTG A L H	GTTTTTATT CAAAAAATAA
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CGTGGAACTC GCACCTTGAG W N S	CTTG GAAC L	ATGCC FACGG C P	TCAC AGTG T	GGAG CCTC E	TCCAACAAAG AGGTTGTTTC S N K A	TCCCGGGAAG AGATGACCAA AGGGCCCTTC TCTACTGGTT S R E E M T K	CTACAAGACC GATGTTCTGG Y K T	TCCG	AGAGTCCCTA ACGCTCGGTT TCTCAGGGAT TGCGAGCCAA
TGGAA LACCTT	TAGCAGCTTG ATCGTCGAAC S S L	CACA GTGT T	CTGAGGTCAC GACTCCAGTG E V T	GCCGCGGGAG CGGCGCCTC P R E	CCAAC SGTTG N	AGATGACCAA TCTACTGGTT M T K	TACAA ATGTT Y K	SATGC STACG	GCTC
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GTGACGGTGT CACTGCCACA V T V S	CTGTGCCCTC GACACGGGAG V P S	TGACAAAACT ACTGITITTGA D K I	TCCCGGACCC AGGGCCTGGG S R T P	GAC F	GTGCAAGGTC CACGTTCCAG C K V	TCCCGGGAAG AGGGCCCTTC S R E E	CGGAGAACAA GCCTCTTGTT E N N	GAACGICITC CITGCAGAAG N V F	agagtcccta Tctcagggat
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ACCG TGGC	AGCGTGGTGA TCGCACCACT S V V T	CTTG	GATC CTAG I	GTGCATAATG CACGTATTAC V H N A	GTACAA CATGTT Y K	CCCA	SCAGC SGTCG Q P	AGGG TCCC	CCCT
CCCCGAACCG GGGGCTTGGC P E P	AGCGTG TCGCAC S V	CAAATCTT STTTAGAA K S C	CTCATGAT GAGTACTZ L M I	GTGCAT CACGTA V H	SCACT CCTCA	CTGCCCCC	ATGGG FACCC	scaccaco corcorco Q Q G	3ACGG CTGCC
	CTCCCTCAGC AGCGTGGTGA GAGGGAGTCG TCGCACCACT S L S S V V T	AAAGTTGAGC CCAAATCTTG TTTCAACTCG GGTTTAGAAC K V E P K S C	CCAAGGACAC CCTCATGATC GGTTCCTGTG GGAGTACTAG K D T L M I	CGGCGTGGAG GTGCATAATG GCCGCACCTC CACGTATTAC G V E V H N A	CTGAATGGCA AGGAGTACAA GACTTACCGT TCCTCATGTT L N G K E Y K	AGGTGTACAC CCTGCCCCA TCCACATGTG GGACGGGGGT V Y I L P P	GTGGGAGGC AATGGGCAGC CACCCTCTCG TTACCCGTCG W E S N G Q P	AAGAGCAGGT GGCAGCAGGG TTCTCGTCCA CCGTCGTCCC K S R W Q Q G	AATAAGCATG CGACGGCCCT TTATTCGTAC GCTGCCGGGA O
AGGACTACTT TCCTGATGAA D Y F	CTCCCTCAGC GAGGGAGTCG S L S	TTGAC	AAGGAC TTCCTG K D	CGGCGTGGAG GCGCACCTC G V E	ATGGCA TACCGT	GTGTACA( CACATGTY V Y T	SCAGA(	AGCAG TCGTC	AGCA
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2101	2201	2301	2401	310	343	2701	2801	2901	3001

3101 TGCGGTAGIT TATCACAGIT AAAITGCTAA CGCAGTCAGG CACCGTGTAT GAAATCTAAC AATGCGCTCA TCGTCATCCT CGGCACCGTC ACCCTGGATG ACGCCATCAA ATAGTGTCAA ITTAACGAIT GCGTCAGTCC GTGGCACATA CTTTAGATTG TTACGCCAGCA AGCAGTAGGA GCCGTGGCAG TGGGACCTAC
^Start Tet Resistance Coding Sequence

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK

Phone No.: 612.371.5311

3201 CTGTAGGCAT AGGCTTGGTT ATGCCGGTAC TGCCGGGCCT CTTGCGGGAT ATCGTCCATT CCGACAGCAT GGCTGATCA TATGGCGTGC TGCTAGCGAA GACATCCGTA GGCTGTCGTA GGCTGTCGTA GGCTGTCGTA GGCTCGCTA ACGGCCCACG ACGATCGCGA

3301

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 35 of 50

AGAGTCGAAT TCTCAGCTTA	CAGGTAGAGG GTCCATCTCC	CTCGTCAGTA GAGCAGTCAT	GTACGCAAGT	CGCTGATATC GCGACTATAG A D I hain^	GGTGATAGCT CCACTATCGA G D S Y	rctctggatc agagacctag S G S	GTACACATTT CATGTGTAAA Y T F	ACTGCCTCTG TGACGGAGAC T A S V	STGTCACAGA CACAGTGTCT V T E
AAAAAGAAGA	GTTGATTGAT (		TTTGTAACTA (	CAAACGCGTA GTTTGCGCAT N A Y IGE light o	CGATTACGAT GGTGATAGCT GCTAATGCTA CCACTATCGA D Y D G D S Y	CCTTCTCGCT TCTCTGGATC GGAAGAGCGA AGAGACCTAG PSRFSGS	ACGAGGATCC IGCTCCTAGG E D P	GAAATCTGGA Z	TCCCAGGAGA GTGTCACAGA AGGGTCCTCT CACAGTGTCT S Q E S V T E
GITGITATIT AAGCITGCCC CAACAATAAA ITCGAACGGG	ACCAACAGCG TGGTTGTCGC	GCGATTACGT AAAGAAGTTA TTGAAGCATC CGCTAATGCA TTTCTTCAAT AACTTCGTAG	GCCGAGACTT ATAGTCGCTT TGTTTTTATT TTTTAATGTA CGGCTCTGAA TATCAGCGAA ACAAAAATAA AAAATTACAT		GTCAGAGCGT CAGTCTCGCA Q S V	GTCTGGAGTC CAGACCTCAG S G V	CAGCAAAGTC GTCGTTTCAG Q Q S H	CCGCCATCTG ATGAGCAGTT GCCGGTAGAC TACTCGTCAA P P S D E Q L	AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCATGTCACC TTCCACCTAT TGCGGGAGGT TAGCCCATTG V Q W K V D N A L Q S G N
GTTGTTATTT CAACAATAAA	GCGCAAAATG CGCGTTTTAC		TGTTTTTATT ACAAAAATAA	GTTCGTTTTT CAAGCAAAA F V F	TGCCGTGCCA OR ACGCCACGT OR ACGCCACGGT OR A S	CGTACCTGGA GCATGGACCT Y L E	TTATTACTGT AATAATGACA Y Y C	CCGCCATCTG GGCGGTAGAC P P S D	AAGGTGGATA ACGCCCTCCA TTCCACCTAT TGCGGGAGGT K V D N A L Q
AAATACAGAC ATGAAAAATC TCATTGCTGA TTTATGTCTG TACTTTTAG AGTAACGACT	CTGCAATGCT TCGCAATATG GACGTTACGA AGCGTTATAC	GAGCTGCTGC CTCGACGACG	ATAGTCGCTT TATCAGCGAA	GAAGAATATC GCATTTCTTC TTGCATCTAT CTTCTTATAG CGTAAGAAG AACGTAGATA K N I A F L L A S M signal TIR-1	CACCATCACC GTGGTAGTGG T I T	ACTACTGATT TACGCGGCCT TGATGACTAA ATGCGCCGGA L L I Y A A S	CAGCCGGAAG ACTTCGCAAC GTCGGCCTTC TGAAGCGTTG Q P E D F A T	CTTCATCTTC GAAGTAGAAG F I F	AAGGTGGATA TTCCACCTAT K V D N
AAATACAGAC ATGAAAAATC TTTATGTCTG TACTTTTAG		CGACGATACG GCTGCTATGC	GCCGAGACTT CGGCTCTGAA	GCATTTCTTC CGTAAAGAAG A F L L	GCATAGGGT GCTATCCCA D R V			ACTGTGGCTG CACCATCTGT TGACACCGAC GTGGTAGACA T V A A P S V	AGTACAGTGG TCATGTCACC V Q W
	ATTATCGTCA TAATAGCAGT	CCCGATGCCA GCATTCCTGA GGGCTACGGT CGTAAGGACT	GCTGTCATAA AGTTGTCACG CGACAGTATT TCAACAGTGC			AAACCAGGAA AAGCTCCGAA TTTGGTCCTT TTCGAGGCTT K P G K A P K	CAGCAGTCTG GTCGTCAGAC S S L		TTCTATCCCA GAGAGGCCAA AAGATAGGGT CTCTCCGGTT F Y P R E A K
TTGGATAAGG AACCTATTCC	AGCTTTGGAG TCGAAACCTC			GAATTATGAA CTTAATACTT M K ^STII	CTCCCTGTCC GAGGGACAGG S L S	AAACCAGGAA TTTGGTCCTT K P G K	CTCTGACCAT GAGACTGGTA L T I	GATCAAACGA CTAGTTTGCT I K R	
GAATTCAACT TCTCCATACT CTTAAGTTGA AGAGGTATGA	GAACTGTGTG CGCAGGTAGA CTTGACACAC GCGTCCATCT	GGGCGCTGTA CGAGGTAAAG CCCGCGACAT GCTCCATTTC	AAAAGTTAAT CTTTTCAACA ITTTCAATTA GAAAAGTTGT	AGGGTATCTA TCCCATAGAT	AGTCCCCGAG TCAGGGGCTC S P S	GTATCAACAG CATAGTTGTC Y Q Q	GCCTACTGGG ACGGATTTTCA GCCAAGACCC TGCCTAAAGT G S G T D F T	GGACAGGTA CCAAGGTGGA CCTGTCCCAT GGTTCCACCT G Q G T K V E	TTGTGTGCCT GCTGAATAAC AACACACGGA CGACTTATTG V C L L N N
1 GAATTCAACT TCTCCATACT CTTAAGTTGA AGAGGTATGA	101 GAACTGTGTG CGCAGGTAGA CTTGACACAC GCGTCCATCT	201 GGGCGCTGTA CGAGGTAAAG CCCGCGACAT GCTCCATTTC	301 AAAAGTTAAT CTTTTCAACA TTTTCAATTA GAAAAGTTGT	401 TCACGTAAAA AGGGTATCTA AGTGCATTTT TCCCATAGAT 1	501 CAGCTGACCC AGTCCCC GTCGACTGGG TCAGGGG 26 Q L T Q S P	601 ACATGAACTG GTATCAACAG TGTACTTGAC CATAGTTGTC 60 M N W Y Q Q	701 CGGTTCTGGG ACGGATTTCA GCCAAGACCC TGCCTAAAGT 93 G S G T D F T		
г	101	201	301	401	501	601	701	801	901

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 36 of 50

AGTCACCCAT TCAGTGGGTA V T H	ACCCGGGGAT TGGGCCCCTA	GATTATCGTC CTAATAGCAG	AGCATTCCTG TCGTAAGGAC	AAGTTGTCAC TTCAACAGTG	AGAAGAATAT TCTTCTTATA K N I Signal TIR	GCAGCCAGGG CGTCGGTCCC Q P G	CTGGAATGGG GACCTTACCC L E W V	TCTACCTGCA AGATGGACGT Y L Q	TCAAGGAACC AGTTCCTTGG Q G T	CTGGGCTGCC GACCCGACGG L G C L
ACGCCTGCGA TGCGGACGCT A C E	CGAGCTCGGT	AAGCTTTGGA	GCCCGATGCC	AGCTGTCATA	AGAATTATGA AGAAGAATAT TCTTAATACT TCTTCTTATA M K K N I ^STII Signal TII	GTGGCCTGGT CACCGGACCA G L V	GGGTAAGGGC CCCATTCCCG G K G	AAAAACACAT TTTTTGTGTA K N T F	CCGTGTGGGG GGCACACCCC V W G	CACAGCGGCC GTGTCGCCGG T A A
CACAAAGTCT GTGTTTCAGA H K V Y	CGCATCGTGG GCGTAGCACC	GCGCAGGTAG CGCGTCCATC	ACGAGGTAAA TGCTCCATTT	TCTTTTCAAC AGAAAAGTTG	AAGGGTATCT TTCCCATAGA	GAGTCTGGCG CTCAGACCGC ESGG Chain	GTCAGGCCCC CAGTCCGGGG	CGACGACTCC GCTGCTGAGG D D S	TGGCACTTCG ACCGTGAAGC W H F A	CCTCTGGGGG GGAGACCCCC S G G
CTACGAGAAA GATGCTCTTT Y E K	CTACGCCGGA GATGCGGCCT	TGAACTGTGT ACTTGACACA	GGGGCGCTGT	AAAAAGTTAA TTTTTCAATT	TTCACGTAAA AAGTGCATTT	TCAGCTGGTG AGTCGACCAC Q L V IgE heavy	AACTGGATCC TTGACCTAGG N W I R	CTATAAGTCG GATATTCAGC I S R	TTTCGGTCAC AAAGCCAGTG F G H	TCCAAGAGCA AGGTTCTCGT S K S T
GCAAAGCAGA CGTTTCGTCT K A D	ATTAAATCCT TAATTTAGGA	GCATCTCGAA CGTAGAGCTT	TCAGGTAGAG AGTCCATCTC	CCTCGTCAGT	AGTACGCAAG TCATGCGTTC	ACGCTGAGGT TGCGACTCCA A E V ^anti	ATATAGCTGG TATATCGACC Y S W	GGCCGTATCA CCGGCATAGT G R I T	GCAGCCACTA CGTCGGTGAT S H Y	GGCACCCTCC CCGTGGGAGG A P S
CTGACGCTGA GACTGCGACT L T L S	GAGAGTGTTA CTCTCACAAT E C O	TTGCCGACGC	GGTTGATTGA CCAACTAACT	ATTGAAGCAT TAACTTCGTA	ATTTGTAACT TAAACATTGA		TCACCTCCGG AGTGGAGGCC T S G	TAGCGTCAAG ATCGCAGTTC S V K	TGTGCTCGAG ACACGAGCTC C A R G	TCTTCCCCCT AGAAGGGGGA F P L
CAGCAGCACC GTCGTCGTGG S S T	TTCAACAGGG AAGTTGTCCC F N R G	TTTTTTATTG AAAAAATAAC	GACCAACAGC	TAAAGAAGTT ATTTCTTCAA	TTGTTTTTAT TTTTTAATGT AACAAAAATA AAAAATTACA	TTCTATTGCT AAGATAACGA S I A	GGCTACTCCA CCGATGAGGT G Y S I	ACTATAACCC TGATATTGGG Y N P	CGTCTATTAT GCAGATAATA V Y Y	GGCCCATCGG CCGGGTAGCC G P S V
CCTACAGCCT GGATGTCGGA Y S L	CACAAAGAGC GTGTTTCTCG T K S	ວອວວວອອວອອ ອວອອອວວອອວວ	GGCGCAAAAT CCGCGTTTTA	CGCGATTACG GCGCTAATGC		TGTTCGTTTT ACAAGCAAAA F V F	TGCAGTTTCT ACGTCAAAGA A V S	GGATCGACTA CCTAGCTGAT G S T N	AGGACACTGC TCCTGTGACG D T A	CTCCACCAAG GAGGTGGTTC S T K
GCAGGACAGC AAGGACAGCA CGTCCTGTCG TTCCTGTCGT Q D S K D S T	CAGGGCCTGA GCTCGCCCGT GTCCCGGACT CGAGCGGGCA Q G L S S P V	CTAGGCCTAA CGCTCGGTTG GATCCGGATT GCGAGCCAAC	ACTGCAATGC TTCGCAATAT TGACGTTACG AAGCGTTATA	GGAGCTGCTG	GGCCGAGACT TATAGTCGCT CCGGCTCTGA ATATCAGCGA	CGCATTICTI CTIGCATCTA GCGTAAAGAA GAACGTAGAT A F L L A S M	GGCTCACTCC GTTTGTCCTG CCGAGTGAGG CAAACAGGAC G S L R L S C	TTGCATCGAT TACGTATGAC AACGTAGCTA ATGCATACTG A S I T Y D	CTGCGTGCTG GACGCACGAC L R A E	CTGGTCACCG TCTCCTCGGC GACCAGTGGC AGAGGAGCCG L V T V S S A
			1301 ACTGCAATGC TTCGCAATAT TGACGTTACG AAGCGTTATA	1401 ACGACGATAC GGAGCTGCTG TGCTGCTATG CCTCGACGAC	1501 GGCCGAGACT TATAGTCGCT CCGGCTCTGA ATATCAGCGA 1				1901 GATGAACAGC CTACTTGTCG 106 M N S	
1001	1101	1201	1301	1401	1501	1601	1701	1801	1901	2001

Inventor: SIMMONS
Docket No.: 11669.120USU1
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Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
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FIG. 20C

CACCTTCCCG GCTGTCCTAC AGTCCTCAGG GTGGAAGGGC CGACAGGATG TCAGGAGTCC T F P A V L Q S S G	GTGAATCACA AGCCCAGCAA CACCAAGGTG CACTTAGTGT TCGGGTCGTT GTGGTTCCAC V N H K P S N T K V	TGGGGGGACC GTCAGTCTTC CTCTTCCCCC ACCCCCTGG CAGTCAGAAG GAGAAGGGGG G G P S V F L F P P	CGAAGACCCT GAGGTCAAGT TCAACTGGTA GCTTCTGGGA CTCCAGTTCA AGTTGACCAT E D P E V K F N W Y	GTGGTCAGCG TCCTCACCGT CCTGCACCAG CACCAGTCGC AGGAGTGGCA GGACGTGGTC V V S V L T V L H Q	CCATCTCCAA AGCCAAAGGG CAGCCCCGAG GGTAGAGGTT TCGGTTTCCC GTCGGGGCTC I S K A K G Q P R E	GGTCAAAGGC TTCTATCCCA GCGACATGGC CCAGTTTCCG AAGATAGGGT CGCTGTAGCG V K G F Y P S D I A	GGCTCCTTCT TCCTCTACAG CAAGCTCACC CCGAGGAAGA AGGAGATGTC GTTCGAGTGG G S F F L Y S K L T	CTGCACAACC ACTACACGCA GAAGAGCCTC TCCCTGTCTC GACGTGTTGG TGATGTGCGT CTTCTCGGAG AGGGACAGAG L H N H Y T Q K S L S L S P	ACTCATGITI GACAGCITAT CATCGAIAAG TGAGTACAAA CTGTCGAATA GTAGCTAITC	CGCTCATCGT CATCCTCGGC ACCGTCACCC GCGAGTAGCA GTAGGAGCCG TGGCAGTGGG
GCGGCGTGCA CGCCGCACGT G V H	CATCTGCAAC GTAGACGTTG I C N	CCTGAACTCC GGACTTGAGG P E L L	ACGTGAGCCA TGCACTCGGT V S H	CACGTACCGT GTGCATGGCA T Y R	ATCGAGAAAA TAGCTCTTTT I E K T	TGACCTGCCT ACTGGACGGA T C L	GGACTCCGAC CCTGAGGCTG D S D		CCGGGCGTTT TTTATTGTTA GGCCCGCAAA AAATAACAAT	GIGIAIGAAA ICIAACAAIG CACAIACIII AGAIIGIIAC
GCCCTGACCA CGGGACTGGT A L T S	CCCAGACCTA GGGTCTGGAT Q T Y	GTGCCCAGCA CACGGGTCGT C P A	GTGGTGGTGG CACCACCC V V V D	AGTACAACAG TCATGTTGTC Y N S	CCCAGCCCCC GGGTCGGGGG	CAGGTCAGCC GTCCAGTCGG Q V S L	CTCCCGTGCT GAGGGCACGA P V L	GCATGAGGCT CGTACTCCGA H E A		GTGTATGAAA CACATACTTT
GAACTCAGGC CTTGAGTCCG N S G	AGCTTGGGCA TCGAACCCGT S L G T	CATGCCCACC GTACGGGTGG C P P	GGTCACATGC CCAGTGTACG V T C	CGGGAGGAGC GCCCTCCTCG R E E Q	ACAAAGCCCT TGTTTCGGGA K A L	GACCAAGAAC CTGGTTCTTG T K N	AAGACCACGC TTCTGGTGCG K T T P	GTCTTCTCAT GCTCCGTGAT CAGAAGAGTA CGAGGCACTA V F S C S V M	TCGGTTGCCG AGCCAACGGC	GTCAGGCACC
CGGTGTCGTG GCCACAGCAC V S W	GCCCTCTAGC CGGGAGATCG P S S	AAAACTCACA TTTTGAGTGT K T H T	GGACCCCTGA CCTGGGGACT T P E	GACAAAGCCG CTGTTTCGGC T K P	AAGGTCTCCA ACAAAGCCCT TTCCAGAGGT TGTTTCGGGA K V S N K A L	GGGAAGAGAT CCCTTCTCTA E E M	GAACAACTAC CTTGTTGATG N N Y	GTCTTCTCAT CAGAAGAGTA V F S C	TCCCTAACGC TCGGTTGCCG AGGGATTGCG AGCCAACGGC	TGCTAACGCA ACGATTGCGT
GAACCGGTGA CTTGGCCACT E P V T		ATCTTGTGAC, AAAACTCACA TAGAACACTG TTTTGAGTGT S C D K T H T	ATGATCTCCC TACTAGAGGG M I S R	ATAATGCCAA TATTACGGTT N A K	GTACAAGTGC CATGTTCACG Y K C	CCCCCATCCC GGGGGTAGGG P P S R	GGCAGCCGGA CCGTCGGCCT Q P E	GCAGGGGAAC CGTCCCCTTG Q G N	GGCCCTAGAG CCGGGATCTC	ACAGTTAAAT TGCTAACGCA TGTCAATTTA ACGATTGCGT
CTACTTCCCC GATGAAGGGG Y F P	ACTCTACTCC CTCAGCAGCG TGGTGACTGT TGAGATGAGG GAGTCGTCGC ACCACTGACA L Y S L S S V V T V				ATGGCAAGGA TACCGTTCCT G K E					
TGGTCAAGGA ACCAGTTCCT V K D	ACTCTACTCC TGAGATGAGG L Y S	GACAAGAAAG TTGAGGCCAA CTGTTCTTTC AACTCGGGTT D K K V E P K	CAAAACCCAA GGACACCCTC GTTTTGGGTT CCTGTGGGAG K P K D T L	CGTGGACGGC GTGGAGGTGC GCACCTGCCG CACCTCCACG V D G V E V H	GACTGGCTGA ATGGCAAGGA CTGACCGACT TACCGTTCCT D W L N G K E	2701 AACCACAGGT GTACACCCTG TTGGTGTCCA CATGTGGGAC 373 P Q V Y T L	CGTCGCAGTCG GAGAGCAATG GCACCTCACC CTCTCGTTAC V E W E S N G	GTGGACAAGA GCAGGTGGCA CACCTGTTCT CGTCCACCGT V D K S R W Q	3001 CGGGTAAATA AGCATGCGAC GCCCATTTAT TCGTACGCTG 473 G K O	CTTTAATGCG GTAGTTTATC GAAATTACGC CATCAAATAG
2101	2201	2301	2401	2501	339	2701	2801	2901	3001	3101

Inventor: SIMMONS

Inventor: SIMMONS
Docket No.: 11669.120USUI
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
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3201 TGGATGCTGT AGGCATAGGC TTGGTTATGC CGGTACTGCC GGGCCTCTTG CGGGATATCG TCCATTCCGA CAGCATCGCC AGTCACTATG GCGTGCTGA ACCTACGACA TCCGTATCCG AACCAATACG GCCATGACGG CCCGGAGAAC GCCCTATAGC AGGTAAGGCT GTCGTAGCGG TCAGTGATAC CGCACGACGA

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 39 of 50

GAAT	AGG	TA AT	HK	υø	нач	ပေဖ	H ø	10 11 -
AGAGTC TCTCAG	CAGGTAG GTCCATC	CTCGTCAG	GTACGCAAG CATGCGTTC	CGCTGATAT GCGACTATA A D I Aain^	GGTGATAGC CCACTATCG G D S	TCTCTGGAT AGAGACCTA S G S	GTACACATT CATGTGTAA Y T F	ACTGCCTCTC TGACGGAGAC T A S V
AAAAAGAAGA TTTTTCTTCT	GTTGATTGAT CAACTAACTA	TTGAAGCATC AACTTCGTAG	TTTGTAACTA AAACATTGAT	ATTGCTA CAAACGCGTA CGCTG; TAACGAT GTTTGCGCAT GCGAC; I A T N A Y A D anti-IgE light chain^	CGATTACGAT GCTAATGCTA D Y <sub>L</sub> D	CCTTCTCGCT GGAAGAGCGA PSRF	ACGAGGATCC TGCTCCTAGG E D P	GAAATCTGGA ACTGCCTCTG CTTTAGACCT TGACGGAGAC K S G T A S V
AAGCTTGCCC TTCGAACGGG	ACCAACAGCG TGGTTGTCGC	AAAGAAGTTA TTTCTTCAAT	TTTTAATGTA AAAATTACAT	TCTATTGCTA AGATAACGAT S I A T anti-	GTCAGAGCGT CAGTCTCGCA Q S V	GTCTGGAGTC CAGACCTCAG S G V	CAGCAAAGTC GTCGTTTCAG Q Q S H	ATGAGCAGTT TACTCGTCAA E Q L
GTTGTTATTT CAACAATAAA	GCGCAAAATG CGCGTTTTAC	GCGATTACGT CGCTAATGCA	TGTTTTTATT ACAAAAATAA	GTTCGTTTTT CAAGCAAAA F V F	TGCCGTGCCA ACGGCACGGT C R A S	CGTACCTGGA GCATGGACCT Y L E	TTATTACTGT AATAATGACA Y Y C	CCGCCATCTG GGCGGTAGAC P P S D
GAATTCAACT TCTCCATACT TTGGATAAGG AAATACAGAC ATGAAAAATC TCATTGCTGA GTTGTTATTT AAGCTTGCCC AAAAAGAAGA AGAGTCGAAT CTTAAGTTGA AGAGGTATGA AACCTATTCC TTTATGTCTG TACTTTTAG AGTAACGACT CAACAATAAA TTCGAACGGG TTTTTCTTCT TCTCAGCTTA	AGCITIGGAG AITAICGICA CIGCAAIGCI ICGCAAIAIG GCGCAAAAIG ACCAACAGCG GIIGAIIGAI CAGGIAGAGG ICGAAACCIC IAAIAGCAGI GACGIIACGA AGCGIIAIAC CGCGIIITAC IGGIIGICGC CAACIAACIA GICCAICICC	CCCGATGCCA GCATTCCTGA CGACGATACG GAGCTGCTGC GCGATTACGT AAAGAAGTTA TTGAAGCATC CTCGTCAGTA GGGCTACGGT CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TTTCTTCAAT AACTTCGTAG GAGCAGTCAT	GCTGTCATAA AGTIGTCACG GCCGAGACTT ATAGTCGCTT TGTTTTTATT TTTTAATGTA TTTGTAACTA GTACGCAAGT CGACAGTATT TCAACAGTGC CGGCTCTGAA TATCAGCGAA ACAAAATAA AAAATTACAT AAACATTGAT CATGCGTTCA	GAATTATGAA GAAGAATATC GCATTTCTTC TTGCATCTAT GTTCGTTTT TCTATTGCTA CAAACGCGTA CGCTGATATCTTCTTATAG CGTAAAGAAG AACGTAGATA CAAGCAAAAA AGATAACGAT GTTTGCGCAT GCGACTATAG M K K N I A F L L A S M F V F S I A T N A Y A D I ASTII signal TIR-1	CTCCCTGTCC GCCTCTGTGG GCGATAGGGT CACCATCACC TGCCGTGCCA GTCAGAGCGT CGATTACGAT GGTGATAGCT GAGGGACAGG CGGAGACACC CGCTATCCCA GTGGTAGTGG ACGGCACGGT CAGTCTCGCA GCTAATGCTA CCACTATCGA S L S A S V G D R V T I T C R A S Q S V D Y $_{\rm L}$ D G D S Y	AAACCAGGAA AAGCTCCGAA ACTACTGATT TACGCGGCCT CGTACCTGGA GTCTGGAGTC CCTTCTCGCT TCTCTGGATC TTTCGAGCTT TGATGACTAA ATGCGCCGGA GCATGGACCT CAGACCTCAG GGAAGAGCGA AGAGACCTAG K P G K A P K L L I Y A A S Y L E S G V P S R F S G S	CGGTTCTGGG ACGGATTTCA CTCTGACCAT CAGCAGTCTG CAGCCGGAAG ACTTCGCAAC TTATTACTGT CAGCAAAGTC ACGAGGATCC GTACACATTT GCCAAGACCC TGCCTAAAGT GAGACTGGTA GTCGTCAGAC GTCGGCCTTC TGAAGCGTTG AATAATGACA GTCGTTTCAG TGCTCCTAGG CATGTGTAAA G S G T D F T L T I S S L Q P E D F A T Y Y C Q Q S H E D P Y T F	GATCAAACGA ACTGTGGCTG CACCATCTGT CTTCATCTTC CCGCCATCTG ATGAGCAGTT GAAATCTGGA ACTGCCTCTG CTAGTTTGCT TGACACCGAC GTGGTAGACA GAGTAGAAG GGCGGTAGAC TACTCGTCAA CTTTAGACCT TGACGGAGAC I K R I V A A P S V F I F P P S D E Q L K S G I A S V
ATGAAAAATC TACTTTTTAG	CTGCAATGCT GACGTTACGA	CGACGATACG GCTGCTATGC	GCCGAGACTT CGGCTCTGAA	GCATTTCTTC CGTAAAGAAG A F L L	GCGATAGGGT CGCTATCCCA D R V	ACTACTGATT TGATGACTAA L L I	CAGCCGGAAG GTCGGCCTTC Q P E D	CACCATCTGT GTGGTAGACA P S V
AAATACAGAC TTTATGTCTG	ATTATCGTCA TAATAGCAGT	GCATTCCTGA CGTAAGGACT	AGTTGTCACG TCAACAGTGC	GAAGAATATC GC CTTCTTATAG CG K N I A signal TIR~1	GCCTCTGTGG CGGAGACACC A S V G	AAGCTCCGAA TTCGAGGCTT A P K	CAGCAGTCTG GTCGTCAGAC S S L	ACTGTGGCTG TGACACCGAC T V A A
TTGGATAAGG AACCTATTCC	AGCTTTGGAG TCGAAACCTC	CCCGATGCCA GGGCTACGGT		GAATTATGAA CTTAATACTT M K ^STII		AAACCAGGAA TTTGGTCCTT K P G K	CTCTGACCAT GAGACTGGTA L T I	GATCAAACGA CTAGTTTGCT I K R
TCTCCATACT AGAGGTATGA	GAACTGTG CGCAGGTAGA CTTGACACAC GCGTCCATCT	GGGCGCTGTA CGAGGTAAAG CCCGCGACAT GCTCCATTTC	aaaagitaat ciittcaaca iittcaaita gaaaagitgi	TCACGTAAAA AGGGTATCTA AGTGCATTTT TCCCATAGAT	CAGCTGACCC AGTCCCCGAG GTCGACTGGG TCAGGGGCTC Q L T Q S P S	ACATGAACTG GTATCAACAG TGTACTTGAC CATAGTTGTC M N W Y Q Q	ACGGATTTCA TGCCTAAAGT T D F T	GGACAGGGTA CCAAGGTGGA CCTGTCCCAT GGTTCCACCT G Q G T K V E
1 GAATICAACT TCTCCATACT TIGGATAAGG AAATACAGAC AIGAAAAATC TCATIGCIGA GTIGTTATIT AAGCIIGCCC AAAAAGAAGA AGAGTGGAAT CTTAAGTIGA AGAGGIAIGA AACCIATICC TTTAIGICIG TACTITITAG AGTAACGACI CAACAATAAA TICGAAGGG ITTTICTICI ICTCAGCITA	101 GAACTGTGTG CGCAGGTAGA CTTGACACAC GCGTCCATCT	201 GGGCGCTGTA CGAGGTAAAG CCCGCGACAT GCTCCATTTC	301 AAAAGTTAAT CTTTTCAACA TTTTCAATTA GAAAAGTTGT	401 TCACGTAAAA AGGGTATCTA AGTGCATTTT TCCCATAGAT 1		• -		801 GGACAGGTA CCAAGGTGGA CCTGTCCCAT GGTTCCACCT 126 G Q G T K V E
<b>-</b>	101	201	301	401	501	601	701	801

901 TIGIGIGCCT GCTGAATAAC TICTATCCCA GAGAGGCCAA AGTACAGIGG AAGGIGGATA ACGCCCTCCA ATCGGGTAAC TCCCAGGAGA GIGICACAGA AACACACGGA CGACTTATIG AAGATAGGGI CICTCCGGIT TCATGICACC TICCACCIAI TGCGGGAGGI TAGCCCATIG AGGGICCTCI CACAGIGICT 160 V C L L N N F Y P R E A K V Q W K V D N A L Q S G N S Q E S V I E

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311

CTTTGGTAGA

GCCCGGACCA

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^anti-IgE Heavy Chain

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AAGATAACGA

GCGTAAAGAA GAACGTAGAT ACAAGCAAAA

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'Heavy Chain FR1=SubgroupII consensus sequence

1601 CGCATTICIT CITGCATCIA TGITCGITIT ITCTATTGCI ACAAACGCGI ACGCICAGGI TCAGCTGCAA GAGICTGGCC CGGGCCTGGI GAAACCAICI

TGTTTGCGCA TGCGAGTCCA AGTCGACGTT CTCAGACCGG

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CAC CAC FRT TAT	TGCC AGCATTCCTG TAGG TCGTAAGGAC TCATA AAGTTGTCAC GTAT TTCAACAGTG TATGA AGAAGAATAT TACT TCTTCTTATA M K K N I STII Signal TIR-1	GCCCGATGCC CGGGCTACGG AGCTGTCATA TCGACAGTAT AGAATTATGA TCTTAATACT M K	GGCGCAAAAT GACCAACAGC GGTTGATTGA TCAGGTAGAG GGGGCGCTGT ACGAGGTAAA GCCCGATGC AGCATTCCTG CCGCGTTTTA CTGGTTGTCG CCAACTAACT AGTCCATCTC CCCCGCGACA TGCTCCATTT CGGGCTACGG TCGTAAGGAC CGCGATTACG TAAAGAAGTT ATTGAAGCAT CCTCGTCAGT AAAAAGTTAA TCTTTTCAAC AGCTGTCATA AAGTTGTCAC GCGCTAATGC ATTTCTTCAA TAACTTCGTA GGAGCAGTCA TTTTTCAATT AGAAAAGTTG TCGACAGTAT TTCAACAGTG TTGTTTTTAT TTTTTAATGT ATTGTAACT AGTACGCAAG TTCACGTAAA AAGGGTATCT AGAATTATGA AGAAGAATAT AACAAAAATA AAAAATTACA TAAACATTGA TCATGCGTTC AAGTGCATTT TTCCCATAGA TCTTAAATACT TCTTCTTATA AACAAAAATA AAAAAATTAC TAAACATTGA TCATGCGTTC AAGTGCATTT TTCCCATAGA TCTTAAATACT TCTTCTTATA	GGGGCGCTGT CCCCGCGACA AAAAAGTTAA TTTTTCAATT TTCACGTAAA AAGTGCATTT	TCAGGTAGAG AGTCCATCTC CCTCGTCAGT GGAGCAGTCA AGTACGCAAG TCATGCGTTC	GGTTGATTGA CCAACTAACT ATTGAAGCAT TAACTTCGTA ATTTGTAACT	GACCAACAGC CTGGTTGTCG TAAAGAAGTT ATTTCTTCAA TTTTTAATGT AAAAATTACA	GGCGCAAAT CCGCGTTTA CGCGATTACG GCGCTAATGC TTGTTTTTAT AACAAAAATA	ACTGCAATGC TTCGCAATAT TGACGITACG AAGCGITATA ACGACGATAC GGAGCTGCTG TGCTGCTATG CCTCGACGAC GGCCGAGACT TATAGTCGCT CCGGCTCTGA ATATCAGCGA	1301 ACTGCAATGC TTCGCAATAT TGACGTTACG AAGCGTTATA 1401 ACGACGATAC GGAGCTGCTG TGCTGCTATG CCTCGACGAC 1501 GGCCGAGACT TATAGTCGCT CCGGCTCTGA ATATCAGCGA	101
cac arg	AAGTTGTC	AGCTGTCATA TCGACAGTAT	TCTTTTCAAC AGAAAAGTTG	AAAAAGTTAA TTTTTCAATT	CCTCGTCAGT GGAGCAGTCA	ATTGAAGCAT TAACTTCGTA	TAAAGAAGTT ATTTCTTCAA	CGCGATTACG GCGCTAATGC	GGAGCTGCTG CCTCGACGAC	ACGACGATAC TGCTGCTATG	-
erc sac	AGCATTCC TCGTAAGG	GCCCGATGCC CGGGCTACGG	ACGAGGTAAA TGCTCCATTT	GGGGCGCTGT	TCAGGTAGAG AGTCCATCTC	GGTTGATTGA CCAACTAACT	GACCAACAGC	GGCGCAAAAT CCGCGTTTTA	TTCGCAATAT AAGCGTTATA	ACTGCAATGC TGACGTTACG	-
FTC FAG	GATTATCC	AAGCTTTGGA TTCGAAACCT	CCGCCGGGCG TITITIATIG TIGCCGACGC GCATCTCGAA TGAACTGTGT GCGCAGGTAG AAGCTITIGGA GATIATCGTC GGCGGCCCGC AAAAAIAAC AACGGCTGCG CGTAGAGCTI ACTIGACACA CGCGTCCAIC TICGAAACCI CTAAIAGCAG	TGAACTGTGT ACTTGACACA	GCATCTCGAA CGTAGAGCTT	TTGCCGACGC	TTTTTTATTG AAAAAATAAC	ວອວວວອອວອອ	CTAGGCCTAA CGCTCGGTTG GATCCGGATT GCGAGCCAAC	1201 CTAGGCCTAA GGCTCGGTTG GATCCGGATT GCGAGCCAAC	-
SAT	ACCCGGGC TGGGCCCC	CGAGCTCGGT GCTCGAGCCA	CACAAAGAGC TICAACAGGG GAGAGIGITA ATIAAAICCI CIACGCCGGA CGCAICGIGG CGAGCICGGI ACCCGGGGAI GIGITICICG AAGIIGICCC CICICACAAI TAAITIAGGA GAIGCGGCCI GCGIAGCACC GCICGAGCCA IGGGCCCTA I K S F N R G E C O	CTACGCCGGA GATGCGGCCT	ATTAAATCCT TAATTTAGGA	GAGAGTGTTA CTCTCACAAT E C O	TTCAACAGGG GAGAGTGTTP AAGTTGTCCC CTCTCACAA1 F N R G E C O	CACAAAGAGC GTGTTTCTCG T K S	CAGGGCCTGA GCTCGCCCGT GTCCCGGACT CGAGCGGGCA Q G L S S P V	1101 CAGGCCTGA GCTCGCCCGT GTCCCGGACT CGAGCGGGCA 226 Q G L S S P V	ч 9
TA .	TCAGTGGG	ACCCIGCGA TGCGGACGCT A C E	CCIALAGCCI CACCAGCACC CIGACOCIGA GLANGGARA CACAMAGICI ACGCAGCGA GAINGTIGGA GACTGCGACT CGTTTCGTCT GATGCTCTTT GTGTTTCAGA TGCGGACGCT TCAGTGGGTA Y S L S S T L T L S K A D Y E K H K V Y A C E V T H	CIACGAGAAA GATGCTCTTT Y E K	GCAAAGCAGA CGTTTCGTCT K A D	CIGACGCIGA GACTGCGACT L T L S	CAGCACC GTCGTCGTGG S S T	GGATGTCGGA Y S L	GCAGGALAGC AAGGALAGCA CGTCCTGTCG TTCCTGTCGT Q D S K D S T	1001 GCAGGACAGC AAGGACAGCA CGTCCTGTCG TTCCTGTCGT 193 Q D S K D S T	<b>⊣</b> ო

AGTTCCTTGG GACCCGACGG CTGGAATGGG GACCTTACCC TTTTTGTGTA AGATGGACGT TCAAGGAACC CIGGGCIGCC ט o AAAAACACAT ccereresees GTGTCGCCGG CCTTGACTTG TACTGTTTCT GGCTACTCCA TCACCTCCGG ATATAGCTGG AACTGGATCC GTCAGGCCCC GGGTAAGGGC CCCATTCCCG CACAGCGGCC GGCACACCCC Ö G R Ø, X ы CAGTCCGGGG CGACGACTCC GCTGCTGAGG TGCCACTTCG ACCGTGAAGC CCTCTGGGGG CCGTGGGAGG AGGTTCTCGT GGAGACCCCC Ø ø Ö Ω H ø S Ω CTATAAGTCG AAAGCCAGTG TTGACCTAGG æ TTTCGGTCAC TCCAAGAGCA GATATTCAGC н Ξ S ഗ v N N × CCGCCATAGT TATATCGACC GGCCGTATCA AGGACACTGC CGTCTATTAT TGTGCTCGAG GCAGCCACTA CGTCGGTGAT GGCACCCTCC Н S Δ, 2 ß × CTCCACCAAG GGCCCATCGG TCTTCCCCCT GGATCGACTA ACTATAACCC TAGCGTCAAG ATCGCAGTTC TCCTGTGACG GCAGATAATA ACACGAGCTC CCGGGTAGCC AGAAGGGGGA AGTGGAGGCC ۷ ۲ A ß [±, ഗ υ CCTAGCTGAT TGATATTGGG CCGATGAGGT Δ, X S z Д × Ö ATGACAAAGA GAGGTGGTTC × S H ഗ G CTGCGTGCTG GACGCACGAC GGAACTGAAC TACGTATGAC ATGCATACTG TCTCCTCGGC AGAGGAGCCG ပ Д Ø H × L R 1701 CAGACTCTCT AACGTAGCTA 1901 GATGAACAGC GACCAGTGGC CTACTTGTCG 2001 CTGGTCACCG GTCTGAGAGA TTGCATCGAT S z > Ø Σ O 1801

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Inventor: SIMMONS

Docket No.: 11669.120USUI
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK

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AGTCCTCAGG TCAGGAGTCC S S G	CACCAAGGTG GTGGTTCCAC T K V	CTCTTCCCCC GAGAAGGGGG L F P P	TCAACTGGTA AGTTGACCAT N W Y	CCTGCACCAG GGACGTGGTC L H Q	CAGCCCCGAG GTCGGGGCTC Q P R E	GCGACATCGC CGCTGTAGCG D I A	CAAGCTCACC GTTCGAGTGG K L T	TCCCTGTCTC AGGGACAGAG S L S P	CATCGATAAG GTAGCTATTC
AGTC TCAG			TCAA AGTT	_	CAGC GTCG		_		CATC
GCTGTCCTAC AGTCCTCAGG CGACAGGATG TCAGGAGTCC A V L Q S S G	GTGAATCACA AGCCCAGCAA CACTTAGTGT TCGGGTCGTT V N H K P S N	GTCAGTCTTC CAGTCAGAAG S V F	GAGGTCAAGT TCAACTGGTA CTCCAGTTCA AGTTGACCAT E V K F N W Y	TCCTCACCGT AGGAGTGGCA L T V	AGCCAAAGGG TCGGTTTCCC A K G	GGTCAAAGGC TTCTATCCCA CCAGTTTCCG AAGATAGGGT V K G F Y P S	GGCTCCTTCT TCCTCTACAG CCGAGGAAGA AGGAGATGTC G S F F L Y S	GAAGAGCCTC CTTCTCGGAG K S L	GACAGCITAT CATCGATAAG CTGTCGAATA GTAGCTAITC
CACCTTCCCG GTGGAAGGGC T F P	GTGAATCACA CACTTAGTGT V N H K	TGGGGGGACC ACCCCCTGG G G P	CGAAGACCCT GCTTCTGGGA E D P	Gregreages recreaeger Caccagrese assacrasea V V S V L T V	CCATCTCCAA GGTAGAGGTT I S K	GGTCAAAGGC TTCTATCCCA CCAGTTTCCG AAGATAGGGT V K G P Y P S	GGCTCCTTCT TCCTCTACAG CCGAGGAAGA AGGAGATGTC G S F F L Y S	ACTACACGCA TGATGTGCGT Y T Q	actcatgitt Tgagtacaaa
GCCCTGACCA GCGGCGTGCA CGGGACTGGT CGCCGCACGT A L T S G V H	CATCTGCAAC GTAGACGTTG I C N	CCTGAACTCC TGGGGGGACC GGACTTGAGG ACCCCCTGG P E L L G G P	GTGGTGGTGG ACGTGAGCCA CACCACCACC TGCACTCGGT V V V D V S H	CACGTACCGT GTGCATGGCA T Y R	ATCGAGAAAA TAGCTCTTTT I E K T	TGACCTGCCT ACTGGACGGA T C L	GGACTCCGAC CCTGAGGCTG D S D	CTGCACAACC ACTACACGCA GACGTGTTGG TGATGTGCGT L H N H Y T Q	TTTATTGTTA ACTCATGTTT AAATAACAAT TGAGTACAAA
	CCCAGACCTA GGGTCTGGAT Q T Y	GTGCCCAGCA CACGGGTCGT C P A		CGGGAGGAGC AGTACAACAG GCCCTCCTCG TCATGTTGTC R E E Q Y N S	CCCAGCCCCC GGGTCGGGGG	CAGGTCAGCC TGACCTGCCT GTCCAGTCGG ACTGGACGGA Q V S L T C L	CTCCCGTGCT GAGGGCACGA P V L	GCATGAGGCT CGTACTCCGA H E A	CCGGGCGTTT GGCCCGCAAA
GAACTCAGGC CTTGAGTCCG N S G	AGCTTGGGCA TCGAACCCGT S L G T	ATCTIGIGAC'AAAACTCACA CATGCCCACC TAGAACACTG TTTTGAGTGT GTACGGGTGG S C D K T H T C P P	GGTCACATGC CCAGTGTACG V T C		AAGGTCTCCA ACAAAGCCCT TTCCAGAGGT TGTTTCGGGA K V S N K A L	GACCAAGAAC CTGGTTCTTG T K N		GTCTTCTCAT GCTCCGTGAT CAGAAGAGTA CGAGGCACTA V F S C S V M	TCGGTTGCCG AGCCAACGGC
CGGTGTCGTG GCCACAGCAC V S W	GCCCTCTAGC CGGGAGATCG PSS	'AAAACTCACA TTTTGAGTGT K T H T	ATGATCTCCC GGACCCCTGA TACTAGAGGG CCTGGGGACT M I S R T P E	GACAAAGCCG CTGTTTCGGC T K P	AAGGTCTCCA TTCCAGAGGT K V S N	CCCCCATCCC GGGAAGAGA GGGGGTAGGG CCCTTCTCTA P P S R E E M	GAACAACTAC CTTGTTGATG N N Y	GTCTTCTCAT CAGAAGAGTA V F S C	GGCCCTAGAG TCCCTAACGC TCGGTTGCCG CCGGGATCTC AGGGATTGCG AGCCAACGGC
GAACCGGTGA CTTGGCCACT E P V T	TGGTGACTGT ACCACTGACA V T V			ATAATGCCAA TATTACGGTT N A K	GTACAAGTGC CATGTTCACG Y K C		GGCAGCCGGA CCGTCGGCCT Q P E	GCAGGGGAAC CGTCCCCTTG Q G N	
ACCAGITCCT GAIGAAGGGG	ACTCTACTCC CTCAGCAGCG TGGTGACTGT TGAGATGAGG GAGTCGTCGC ACCACTGACA L Y S L S S V V T V	GACAAGAAAG TTGAGCCCAA CTGTTCTTTC AACTCGGGTT D K K V E P K	CAAAACCCAA GGACACCCTC GTTTTGGGTT CCTGTGGGAG K P K D T L	CGTGGACGGC GTGGAGGTGC ATAATGCCAA GCACCTGCCG CACCTCCACG TATTACGGTT V D G V E V H N A K	GACTGGCTGA ATGGCAAGGA CTGACCGACT TACCGTTCCT D W L N G K E	2701 AACCACAGGT GTACACCCTG TTGGTGTCCA CATGTGGGAC 373 P Q V Y T L	CGTGGAGTGG GAGAGCAATG GGCAGCCGGA GCACCTCACC CTCTCGTTAC CCGTCGGCCT V E W E S N G Q P E	GTGGACAAGA GCAGGTGGCA CACCTGTTCT CGTCCACCGT V D K S R W Q	CGGGTAAATA AGCATGCGAC GCCCATTTAT TCGTACGCTG G K O
					GACTGGCTGA ATGGCAAG CTGACCGACT TACCGTTC D W L N G K	AACCACAGGT TTGGTGTCCA P Q V	CGTGGAGTGG GCACCTCACC V E W	GTGGACAAGA CACCTGTTCT V D K S	CGGGTAAATA GCCCATTTAT G K O
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3101 CITITAATGCG GTAGITITATC ACAGITAAAT TGCTAACGCA GTCAGGCACC GTGIATGAAA TCTAACAAIG CGCTCATCGT CATCCTCGGC ACCGTCACCC GAAAITACGC CATCAAAITA GCAGITAA ACGATTGCT CAGTCCGTG CACATACTIT AGATTGTTAC GCGAGIAGCA GTAGGAGCCG TGGCAGTGGG

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311
Sheet 42 of 50

3201 TGGATGCTGT AGGCATAGGC TTGGTTATGC CGGTACTGCC GGGCCTCTTG CGGGATATCG TCCATTCGGA CAGCATCGCC AGTCACTATG GCGTGCTGCT ACCTACGACA TCCGTATCG AACCAATAAC GCCATGAAGC CCCGGAGAAC GCCCTATAGC AGGTAAGGCT GTCGTAGCGG TCAGTGATAC CGCACGACGA

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Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK

Sheet 43 of 50

TACT TICGATAAGG AAATACAGAC AIGAAAATC TCATIGCIGA GIIGITAITI AAGCIIGCCC AAAAAGAAGA AGAGICGAAI	ANGA AACCTAINCC ITIANGICIG TACTITITIAG AGTAACGACI CAACAATAAA TICGAACGGG ITITICITCI ICICAGCITA
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1 GAATTCAACT	CTTAAGTTGA

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GAGCAGTCAT CTCGTCAGTA GCATTCCTGA CGACGATACG GAGCTGCTGC GCGATTACGT AAAGAAGTTA TTGAAGGATC CGTAAGGACT GCTGCTATGC CTCGACGACG CGCTAATGCA TTTCTTCAAT AACTTCGTAG CCCGATGCCA CGAGGTAAAG GGCCCCTGTA CCCGCGACAT

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GAATTATGAA GAAGAATATC GCATTTCTTC TTGCATCTAT GITCGTTTTT TCTATTGCTA CAAACGCGTA CGCTGATATC CITCITATAG CGIAAAGAAG AACGIAGAIA CAAGCAAAAA AGAIAACGAI GITTGCGCAI GCGACTAIAG anti-VEGF Light Chain (1st generation)^ A S M ı L A STII Signal TIR -1 K N I AGTGCATTTT TCCCATAGAT CTTAATACTT X TCACGTAAAA AGGGTATCTA

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Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK

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Phone No.: 612.371.5311

Sheet 44 of 50

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Inventor: SIMMONS

Docket No.: 11669.120USUI
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE

Attorney Name: KATHERINE M. KOWALCHYK

Phone No.: 612.371.5311 Sheet 45 of 50

ACCAGCGGCG TGCACACTT CCCGGCTGTC CTACAGTCCT CAGGACTCTA TGGTCGCCGC ACGTGTGGAA GGGCCGACAG GATGTCAGGA GTCCTGAGAT T S G V H T F P A V L Q S S G L Y	TIG CAACGTGAAT CACAAGCCCA GCAACACAA GGTGGACAAG SAC GTTGCACTTA GTGTTCGGGT CGTTGTGGTT CCACCTGTTC C N V N H K P S N T K V D K	ha ctcctggggg gaccgtcagt cttcctcttc cccccaaaac :rt gaggacccc ctggcagtca gaaggagag gggggtttg : l l g g p s v f l f p p K p	CT CGGTGCTTCT GGGACTCCAG TTCAACT GGTACGTGGA CT CGGTGCTTCT GGGACTCCAG TTCAAGTTGA CCATGCACCT S H E D P E V K F N W Y V D	FIA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG	AGGCAGCCC CGAGAACCAC TC TTTTGGTAGA GGTTTCGGTT TCCCGTCGGG GCTCTTGGTG S K T I S K A K G Q P R E P Q	AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA TCGCCGTGGA TCGGACTGGA CGGACCAGTT TCCGAAGATA GGGTCGCTGT AGCGGCACCT S L T C L V K G F Y P S D I A V E	TC CGACGGCTCC TTCTTCCTCT ACAGCAAGCT CACCGTGGACAG GCCCCAGG AAGAAGGAGA TGTCGTTCGA GTGGCACCTG S D G S F F L Y S K L T V D	AC AACCACTACA CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA TG TTGGTGATGT GCGTCTTCTC GGAGAGGGAC AGAGGCCCAT I N H Y T Q K S L S L S P G K	NTT GTTAACTCAT GTTTGACAGC TTATCATCGA TAAGCTTTAA AA CAATTGAGTA CAAACTGTCG AATAGTAGCT ATTCGAAATT	GADATCTAAC AATGCGCTCA TCGTCATCCT CGGCACCGTC ACCCTGGATG CTTTAGATTG TTACGCGAGT AGCAGTAGGA GCCGTGGCAG TGGGACCTAC	ATCGTCCATT CCGACAGCAT CGCCAGTCAC TATGGCGTGC TGCTAGCGCT TAGCAGGTAA GGCTGTCAGTG ATACCGCACG ACGATCGCGA
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GTGACGGTGT CACTGCCACA V T V S	CTGTGCCCTC GACACGGGAG V P S	TGACAAAACT ACTGITITIGA D K T	TCCCGGACCC AGGGCCTGGG S R T P	CCAAGACAAA GGTTCTGTTT K T K	GTGCAAGGTC CACGTTCCAG C K V		CGGAGAACAA GCCTCTTGTT E N N	GAACGTCTTC CTTGCAGAAG N V F		AAATTGCTAA TTTAACGATT	ATGCCGGTAC TACGGCCATG
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Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311

Sheet 46 of 50

>Anti-VEGF (VNERK version) IgG1 Expression Cassette with heavy chain FR1,2,3,4=consensus subgroup!

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CTTCACCTCC TCTCTCCACT CTGGAGTCCC

GCTCCGAAAG TACTGATTTA CGAGGCTTTC ATGACTAAAT

ACCAGGAAAA TOGTCCTTTT

601 ATCHACAGAA TAGTTGTCTT

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GAAGTGGAGG AGAGAGGTGA F T S S L H S

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AAGAGCGAAG AGACCTAGGC S R F S G S G

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE

Sheet 47 of 50

kpnī

asp718 ACAGIATAGC ACCGIGCCGI GGACGITIGG ACAGGGIACC TOTCCCATGG acc651 ø TGCCACGCCA CCTGCAAACC Ö 3 > TGTCATATCG တ ø TTCGCAACTT ATTACTGTCA AAGCGTTGAA TAATGACAGT O U COGTCTTCTG GCCAGAAGAC bpuai bbsi Ω Ø Q, **GCAGTCTGCA** COTCAGACGT pstl u o Ø CTGACCATCA GACTGGTAGT H 701 GOATITICACT

CACACGGACG grandcerac U AATCIGGAAC IGCIICIGIT TTAGACCTIG ACGAAGACAA ഗ asp700 GAGCAGTTGA CTCGTCAACT o 臼 GCCATCTGAT CGGTAGACTA Ω ഗ Д CCATCTOTOT TCATCTTCCC AGTAGAAGGG Ω Ç4 н **bpuAI** GOTAGACAGA Isdd TOTOGCTGCA ACACCGACGT begi TCAAACGAAC AGTITIGCTIG TICCACCICI 801 AAGGTGGAGA CCTAAAGTGA D4

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GTCACAGAGC AGGACAGCAA CAGTGTCTCG TCCTGTCGTT O CCAGGAGAGT GGTCCTCTCA ഗ Ø œ GAGGCCAAAG TACAGTGGAA GGTGGATAAC GCCCTCCAAT CGGGTAACTC CGGGAGGTTA GCCCATTGAG ຜ z U O ы CCACCTATTG ATGTCACCTT CTCCGGTTTC CTATCCCAGA GATAGGGTCT ACTITATIONA TGAATAACTT asp700 X 160 901

BBtI Ω > X o × A B ø, × Z

CCCGGACTCG CAAAGICIAC GCCIGCGAAG ICACCCAICA GGGCCIGAGC alw261/bsmAI CGGACGCTTC AGTGGGTAGT O = > Ø GTTTCAGATG > × CACGCTCAGC AAAGCAGACT ACGAGAACA TGCTCTTTGT × Œ CTGCGACTCG TTTCGTCTGA Δ blpI/bpull02I X A **GCAGCACCCT** COTCOTOGGA TACAGCCTCA ATGTCGGAGT 1001 GCACCACCACC cerencered

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QAGTGTTAAT TAAATCCTCT ACGCCGGACG CATCGTGGCG AGCTCGGTAC CCGGGGATCT AGGCCTAACG TECEGCCTEC GIAGCACCEC ICGAGCCATE GECCCTAGA ICCGGATIGC stuI smal ecl136II CTCACAATTA ATTTAGGAGA paci U M CAACAGGGGA GITGICCCCT O æ z 1101 TOGCCCGTCA CAAAGAGCTT GITICICGAA Œ Ø AGCGGGCAGT > 8 226

start lambda to terminator^

avill/fspl hindill bapMI

TITIAITGIT GCCGACGCG AICICGAAIG AACIGIGIGC GCAGGIAGAA GCITIGGAGA ITAICGICAC IGCAAIGCIT CGGCTGCGCG TAGAGCTTAC TTGACACACG CGTCCATCTT CGAAACCTCT AATAGCAGTG ACGTTACGAA GAGCCAACGG CGGCCCGCAA AAAATAACAA CICGGITGCC GCCGGGCGIT

\*end lambda to terminator

Inventor: SIMMONS
Docket No.: 11669.120USU1
Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING
YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE
Attorney Name: KATHERINE M. KOWALCHYK
Phone No.: 612.371.5311

ahdI/eam1105I eagl/xmalll/eclXI GCGTTATACC GCGTTTTACT GGTTGTCGCC AACTAACTAG TCCATCTCCC CCGCGACATG CTCCATTTCG GGCTACGGTC GTAAGGACTG CTGCTATGCC CCAACAGGGG TTGATTGATC AGGTAGAGGG GGCGCTGTAC GAGGTAAAGC CCGATGCCAG CATTCCTGAC GACGATACGG pwil COCAAAATGA 1301 COCMATATOG

Dami

Keli

1401 AGCIGCIGCG CGAITACGIA AAGAAGIIAI IGAAGCAICC ICGICAGIAA AAAGIIAAIC ITITCAACAG CIGICAIAAA GIIGICACGG CCGAGACIIA ICGACGAC GCIAAIGCAI ITCITCAAIA ACTICGIAGG AGCAGICAII IITCAAITAG AAAAGIIGIC GACAGIAIII CAACAGIGCC GGCTCIGAAI

atcagcgaaa caaaaataaa aaattacata aacattgatc atgcgttcaa gtgcattttt cccatagatc ttaatacttc ttcttatagc gtaaagaaga **GITITITATIT ITTAATGIAF ITGIAACTAG** TACGCAAGTI CACGTAAAAA GGG**TATCTAG AATTATGAA**G AAGAATATCG **CAITICITC**I KNIX \*STII Signal TIR-1 xbal gpel 1501 TAGTOGCTTT

Q V Q L V Q S G A B V K K P G A S V K ^ start xVEGF(VNERK) heavy chain (consensus subgroup I framework GTCTGGCGCA GAGGTGAAAA AGCCAGGGGC TTCAGTTAAA acstroriae aageraaraa gataacgang tingegeang cgagteerag tegaeeregt eagaeegegt eteeretitt teggteeeeg aagneaaith TICOTITITI CIAINGCIAC AAACGCGIAC GCICAGGIIC AGCIGGIGCA bad IImd beiWI/spli NAY mlui > 1601 TECATCTATE တ

CININCCITC ACCANCIATO GIAINAACIG GGICCGICAG GCCCCGGGIC AGGGCCTGGA AIGGAIGGGA IGGAIIAACA GATATGGAAG TGGTTGATAC CATATTTGAC CCAGGCAGTC CGGGGCCCAG TCCCGGACCT TACCTACCT ACCTAATTGT z alw261/bsmAI η Π xmaI/pspAI P . G . Q BMAI æ X V R \*FR1=SubgroupI z H z H GENERAL ANGCITCEGG CATAGGACAT TTCGAAGACC hindill တ 43

ACTIGGCIGG ALACGACGC TAAAGTITGC AGCACAATGA TAGTGACGAC TGTGGAGGTG ATCGTGTCGT ATGTACCTTG ACAGATCGGA TGAACCGACC TATGCTGCGG ATTTCAAACG TCGTGTTACT ATCACTGCTG ACACCTCCAC TAGCACAGCA TACATGGAAC TGTCTAGCCT ^FR3=subgroupI 1801 CCIMIACCGG GGATATGGCC agel ×

^FR2=SubgroupI

GGGTCAAGGA CCCAGTTCCT GACACTOCCO TCTATTACTO TOCACOTTAC CCGCACTATT ATGTGAACGA GCGGAAGAGC CACTGGTATT TCGACGTCTG CIGINAACGGC AGAIAAIGAC ACGIGCAAIG GGCGIGAIAA IACACIIGCI CGCCIICICG GIGACCAIAA AGCIGCAGAC aatlI FR4= same sequence for subgroupI, II and III H W Y F bsrBI earI/ksp632I V N E N N N × × H alw44I/snoI **>** 4 · H GOCCICICAG COCCAGACTC S 110

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2001		bseRI esp31 bstEII bsmBI accredrca ccorrecte adcercear regarcat adcadagaa cceaagara ireceara	pspomi/b apai ggcctccacc aagggcccat ccggaggtgg trcccggta a s t k g p s	pspomi/bsp1201 apai bbs: AAGGGCCCAT CGGTC TTCCCGGGTA GCCAG	bpuai spl20i bbsi ccGTCTTCCC GCCAGAAGGG		eri Tectecaaga Aggaggtet S S K S		GGGCACAGCG CCCGTGTCGC G T A	GCCTGGGCT CGGGACCCGA A L G C	-
2101	econi GCCTGGTCAA CGGACCAGTI 7 L V K		tthiii/a agel pflFi ggactactic cccgaaccgg tgacggtgtc ccrgatgaag gggctiggcc actgccacag D Y P P B P V T V S	tthilis/aspi pflFi TGACGGTGTC GT ACTGCCACAG CA T V S W	BPI GTGGAACTCA CACCTTGAGT W N S	hari kasi ehei GGGGCCCTGA CCGCGGGACT G A L T	aps alw ccaccecor ecreeceda s o s		eco8 beu3 ccgcTGTCC TACAGTCCTC GGCCGACAGG ATGTCAGGAG	eco811 bsu361 TACAGTCCTC ATGTCAGGAG	eco811 bsu361/mst11/sau CCTC GGAG
2201	1 AGGACTCTAC TCCTGAGATG 0 G L Y	AGGACTCTAC TCCCTCAGCA GCGTGGTGAC TGTGCCCTCT TCCTGAGATG AGGGAGTCGT CGCACCACTG ACACGGAGAGAGA	GCGTGGTGAC CGCACCACTG V V T	TGTGCCCTCT ACACGGGAGA V P S		GCACCCAGAC CGTGGGTCTG T Q T	CTACATCTGC GATGTAGACG Y I C	AACGTGAATC TTGCACTTAG N V N H	ACAAGCCCAG TGTTCGGGTC K P S	CAACACCAAG GITGIGGITC N I K	
2301		AAGTTGAGCC TTCAACTCGG V B P	CAAATCTIGT GITIAGAACA KSC	CTOTITICAG D K T H	ACACATGCCC TGTGTACGGG T C P	alwNI alw26 ACCGTGCCCA GC TGGCACGGGT CG	NI 261/bsmAI GCACCTGAAC CGTGGACTTG A P E L	ah TCCTGGGGGG AGGACCCCC	bpuAI bbsI ahdI/eam1105I earI/ks TCCTGGGGGG ACGTCAGTC TTCCTCTTCC AGGACCCCC TGGCAGTCAG AAGGAGAAGG	eari/ksp6321 TTCCTCTTCC AAGGAGAAGG P L P P P L P P P L P P P P P P P P P P	Docket N Title: M YIELD C Attorney
2401	2401 CCCCAAAACC GGGGTTTTGG 277 P K P	CAAGGACACC GTTCCTGTGG K D T		rcal ecc baphi bar crcardarcr cccgdacccc Gagracraga gggccrgggg	0.5	GTGGTGG CACCACC V V V	btrI TGGACGTGAG ACCTGCACTC D V S	drdi bpuAI bbsI CCACGAAGAC GGTGCTTCTG H E D	eco811 bsu361/mstII/sauI ccrgaggrca AgrrcaAcrg ggacrccagr rcaagrraac	I/saul AGTTCAACTG TCAAGTTGAC F N W	SIMMONS No.: 11669.120USU1 ETHODS FOR PRODUCIN OF ANTIBODIES OR ANTI Name: KATHERINE M. K o.: 612.371.5311 of 50
2501		GIACGIGGAC GGCGIGGAGG IGCAIAAIGC CAIGCACCIG CCGCACCICC ACGIAIIACG Y V D G V B 'V H N A	OGCOTGOAGO TOCATAATOC CCGCACCTCC ACGTATTACO G V B V H N A	CAAGACAAAG GTTCTGTTTC K T K		Bacil/sstilkspi bseri CCGCGGGAGG AGCAGTACAA GGCGCCTCC TCGTCATGTT PREEQYN	CAGCACGTAC GTCGTGCATG S T Y	CGTGTGGTCA GGGTCCTCAC GCACCAGT CGCAGGAGTG R V V S V L T		begi econi corccrocac ocagaacoro v l h	IGEN BINDING FRAGN
2601	1 CAGGACTGGC GTCCTGACCG 3 Q D W L	TGAATGGCAA ACTTACCGTT N G K	DBAI GGAGTACAAG TGCAAGGTCT CCTCATGTTC ACGTTCCAGA E Y K C K V S	DBAI TGCAAGGTCT ACGTTCCAGA C K V S	CCAACAAAGC GGTTGTTTCG N K A	CCTCCCAGCC GGAGGGTCGG L P A	CCCATCGAGA GGGTAGCTCT P I B K		CAAAGCCAAA GTTTCGGTTT K A K	GGCAGCCCC CCCGTCGGGG G Q P R	BODIES AND IMP MENTS IN CELL C
			••		FI	FIG. 23D					ROVING ULTURE

Title: METHODS FOR PRODUCING HUMANIZED ANTIBODIES AND IMPROVING YIELD OF ANTIBODIES OR ANTIGEN BINDING FRAGMENTS IN CELL CULTURE Attorney Name: KATHERINE M. KOWALCHYK Phone No.: 612.371.5311

clai/bsp106 CTGCCGAGGA AGAAGGAGAT GTCGTTCGAG GCAGAAGAGC CTCTCCCTGT CGTCTTCTCG GAGAGGACA TATCATCGAT TCTTCCTCTA CAGCAAGCTC TATTOGTACG CTGCCGGGAT CTCAGGGATT GCGAGCCAAC GGCGGCCCGC AAAAATAAC AATTGAGTAC AAACTGTCGA ATAGTAGCTA CCAGCGACAT GOTCGCTOTA bapDI Δ earl/ksp632I Ø Ø TITITITATIG TIAACTCATG TITGACAGCT GGCTTCTATC CCGAAGATAG S > sapī H × Œ Ö GATGCATGAG GCTCTGCACA ACCACTACAC GACGGCTCCT CTACGTACTC CGAGACGTGT TGGTGATGTG CCTGGTCAAA GGACCAGTTT ß > > O Ħ hpal AACCAGGTCA GCCTGACCTG CGGACTGGAC GCTGGACTCC CGACCTGAGG bapMI U Ø ä nsil/avalli bsgl Δ H CACGGCCTA CACTCCTAA CGCTCGGTTG CCGCCGGGCG GCGGAGGGCA TTGGTCCAGT ഗ TOGORGAGCA ATOGOCAGCC GORGAACAAC TACAAGACCA CGCCTCCCGT > > ppu10I Δ, H **BexAI** O Д Σ CCTCTTGTTG ATGTTCTGGT CATGCTCCGT GTACGAGGCA CCCGGGAAGA GATGACCAAG CTACTGGTTC > Н Ø smal earl/ksp6321 H × U Σ × GCAGCAGGGG AACGTCTTCT TTGCAGAAGA CCACATGTGG GACGGGGGTA GGGCCCTTCT xmaI/pspAI z ppual rem Ibbsi asp700 > œ M z COTOOTCCCC ACCURCACOT TACCCOTOGG GGTGTACACC CTGCCCCCAT Ø A Q, ø O bep14071/berGI Q, O ø berdi CTCCGGGTAA ATAAGCATGC 2901 ACCOTGGACA AGAGCAGGTG TCTCGTCCAC aphI **DepMI** œ ۲ ۸ M GAGGCCCATT TOGCACCTOT 2701 GAGAACCACA **accacator** COCCOTGGAG CICITOGIGI Ω > > 4 H 3001 2801 410 443 377

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AAGCTITAAT GCGGIAGITT AICACAGITA AATIGCIAAC GCAGICAGGC ACCGIGIAIG AAAICIAACA AIGCGCICAI CGICAICCIC GGCACCGICA TICGARATTA COCCATCARA INGIGICART ITRACGATTO CGICAGICCG IGGCACATAC ITTAGAITGI IACGCGAGIA GCAGIAGGAG CCOTGGCAGI 3101

CCCTGGAIGC TGIAGGCAIA GGCITGGIIA TGCCGGIACI GCCGGGCCTC TIGCGGGAIA TCGICCAITC CGACAGCAIC GCCAGICACI AIGGCGIGCI GOGACCTACG ACATCOGTAT COGAACCAAT ACGGCCATGA CGGCCCGGAG AACGCCCTAT AGCAGGTAAG GCTGTCGTAG CGGTCAGTGA TACCGCACGA 3201

ecoRV

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> length: 3300

1806 2126 795 1176 accest (GGTACC): satii (GACGTC): ageI (ACCGGT):

346 1495 2380 590 ALV261 (CAGNINICTG): ahdi (GACMMMNGTC) aballi (TTTAAA):

alw44I (GTGCAC):